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PLANT-TISSUE RELATIONS OF THE SUGAR-BEET CURLY-TOP VIRUS[1]

By C. W. BENNETT

Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The distribution of virus in different organs of affected plants received attention from some of the pioneer investigators in the field of plant virus diseases. More recently, consideration has been given to the tissues that may be concerned in the increase and distribution of virus in the plant and to the production of primary and secondary pathologic symptoms. Enough evidence has been accumulated to indicate a wide range of variability among viruses in their relation to various tissues of affected plants.

The virus of true tobacco mosaic furnishes one of the best examples of rapid and extensive invasion of tissues. It seems to have a general systemic distribution in tobacco (*Nicotiana tabacum* L.) and probably invades nearly all the living cells of the plant. Certain other viruses have a more limited distribution and seem able to invade only specific tissues or parts. For example, it is doubtful whether the curl virus of raspberry (*Rubus trigosus* Michx.) occurs in tissue other than phloem, and the virus of the phony disease of peach (*Amygdalus persica* L.) is known to be restricted to the root system in the peach, though distinct pathologic symptoms occur on the tops of affected plants.

Considerable evidence, largely circumstantial, has been accumulated which indicates an intimate relationship between viruses and phloem tissue, and which may be summarized as follows: (1) In virus diseases, such as potato leaf roll and sugar-beet curly top, in which necrotic areas are characteristic, necrosis is largely restricted to the phloem where it begins; (2) in certain virus diseases, notably tobacco mosaic, sugar-beet curly top, and maize streak, the rate of virus spread in the plant is best explained by assuming that the phloem is the main channel of movement; (3) it is believed by some workers that most insect vectors habitually feed on vascular tissue, and this has been shown to be true in the case of the vector of sugarcane mosaic; (4) the virus of raspberry curl and that of two types of raspberry mosaic may be restricted in their movement through the plant by removing rings of bark. Furthermore, in curly top and certain other virus diseases the low percentage of infection obtained by artificial methods of inoculation may be due to inability, with the relatively crude technic available, to place the virus in susceptible vascular tissue without causing injury that inhibits development of the

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virus. Some of the evidence indicates the possibility of complete restriction of virus to the phloem tissue in a few diseases, although it cannot be contended that this may prove to have a general application.

The wide botanical range of plants affected by the curly-top virus offers possibilities for an extensive study of some of the relations of this virus to different plant tissues. Taking advantage of this fact, experiments have been performed with a view to securing data on such problems as that of determining the tissues in which the virus must be placed to produce infection, the tissues on which the vector of the virus of curly top feeds, the tissues from which the virus may be recovered, and the channels and rate of dispersion of the virus in the plant.

ARTIFICIAL INOCULATION

Infection of sugar beets (*Beta vulgaris* L.) or of other plants susceptible to curly top by other means than the feeding of leaf hoppers, *Eutettix tenellus* (Baker), has been obtained with difficulty and in only a very small percentage of the plants inoculated. Severin (*22*) [2] induced infection in beets by making repeated punctures with insect pins into the crown through drops of expressed beet juice. Carsner and Stahl (*8*) were successful in obtaining infection in only a few of a large number of plants inoculated by artificial means. Dana (*11*) in one experiment produced infection in 8 of 16 beet plants. In other trials in which beets, spinach (*Spinacia oleracea* L.), and tomato (*Lycopersicon esculentum* Mill.) were inoculated, a low percentage of infection was obtained.

An effective method of artificial transmission of curly top would facilitate materially the study of many of the problems presented by this disease and its causal agent. The results of artificial methods of inoculation, whether or not successful in producing infection, should throw some light on the general question of the plant tissues in which the virus may multiply and from which it can exert its effects on the plant as a whole. With these points in mind, a number of methods of inoculation were tried, most of which are already in general use.

The plants inoculated included sugar beet, Hubbard squash (*Cucurbita maxima* Duchesne), Turkish tobacco (*Nicotiana tabacum* L.), and Black Valentine bean (*Phaseolus vulgaris* L.). Affected specimens of all these plants and also macerated beet leaf hoppers were used as sources of inoculum. Plants of various ages and conditions of growth were used with the different methods of inoculation. The experiments were made at Riverside, Calif., from 1929 to 1932.

NEGATIVE RESULTS OF INOCULATIONS THROUGH XYLEM

In the earlier experiments attempts were made to infect through the xylem elements of the vascular bundles. Twenty plants having roots approximately 1 inch in diameter were taken from the soil, the lower third of the main root was cut away, and the cut surface of the remaining root was placed in centrifugalized juice from diseased beets. The plants were placed for 8 hours in a dry atmosphere, to increase transpiration, and were then transplanted to 6-inch pots. All remained healthy.

[2] Reference is made by number (italic) to Literature Cited, p. 700.

In a modification of the above experiment, 10 beets were transplanted to 3-inch pots, a portion of the main root of each beet being allowed to project through the drainage hole at the bottom of the pot. The 3-inch pots were set on the surface of soil in 6-inch pots with the projecting root embedded in the soil of the larger pot. After the plants had become adjusted to this new condition the smaller pot, with the projecting root system, was carefully removed from the 6-inch pot, and the exposed roots were washed free from soil and severely pruned. The 3-inch pots were then placed over containers so that the exposed part of the beet root was immersed in a liquid composed of 1 part juice from diseased beets and 3 parts tap water. The soil in the pots was allowed to become quite dry, and the plants were placed in a dry atmosphere to increase the amount of inoculum taken up by the root system. After 48 hours the plants were removed and transplanted to 6-inch pots. No disease developed in any of these plants.

In a later experiment, 20 rapidly growing beets having a crown diameter of about 1 inch were used. A hole was bored through the crown by means of a small-size cork borer. Glass tubing was inserted to a distance of about one fourth of an inch in each end of this hole. The beets were then joined in series by means of rubber tubing and connected to a liter flask containing centrifugalized juice from diseased beets. A layer of heavy oil was poured over the surface of the beet juice in the flask to reduce oxidation. A gravity flow of beet juice through the system was started, the juice being taken from near the bottom of the flask. The flow of beet juice was regulated to about 20 drops per minute by means of a pinchcock at the distal end of the system. This volume of flow was continued for 48 hours, with a change to fresh beet juice every 12 hours. None of these plants developed signs of curly top.

In the experiments described above it is reasonable to conclude that considerable beet juice was taken up by the tracheae of the inoculated plants and that this juice contained active virus. The failure to produce disease indicates that the curly-top virus does not pass from tracheae into cells or tissues that permit it to become established and to initiate pathologic symptoms.

RESULTS OF VARIOUS METHODS OF INOCULATION

Many plants have been inoculated by other methods. These methods consisted of puncturing leaves, cotyledons, and crowns of young plants through drops of inoculum by means of small needles; rubbing leaves with rolls of cheesecloth saturated with inoculum; and injecting inoculum into the hollow stems of squash and into the pith of tobacco by means of a hypodermic needle.

Inoculum was prepared in the following ways: (1) Diseased plants were ground in a meat chopper, the juice was expressed and centrifugalized, and the relatively clear liquid was decanted and used as inoculum; (2) viruliferous beet leaf hoppers were macerated in a small amount of water in a mortar and used as inoculum; (3) the surfaces of the crowns of diseased beets were cut away with a sharp knife and the exudate from the cut surface was collected and used as inoculum.

The results of these inoculations are given in table 1. Centrifugalized juice from diseased plants proved to be a very poor source of infectious material with the methods of inoculation employed.

Macerated leaf hoppers likewise were a poor source of infectious material, although the number of plants inoculated was too small to justify final conclusions. The best results were obtained from the use of exudate from the cut surface of diseased beets. With this material, 14 of the 124 plants inoculated became infected. Although this is a low percentage of infection, it is so much higher than that obtained by the use of expressed juice that it is worthy of further trial.

HOW THE BEET LEAF HOPPER FEEDS

With few exceptions, insects that are important vectors of plant viruses have mouth parts adapted for sucking plant juices. The feeding habits of a considerable number of species of sucking insects have been studied by several investigators who have determined the relation of feeding punctures to specific tissues. These investigations have dealt predominantly with insects not associated with the spread of plant viruses, though several of the species cause severe injury to their host plants as a result of the introduction of toxic substances.

TABLE 1.—*Results of artificial inoculation of sugar beet, Turkish tobacco, Hubbard squash, and Black Valentine bean with virus from different sources*

Inoculum	Plant inoculated	Method of inoculation [a]	Number of plants	
			Inoculated	Infected
Juice of beet	Beet	1	80	0
Do	Hubbard squash	1, 2	80	0
Do	do	4	20	0
Juice of Hubbard squash	Beet	2	80	0
Do	do	1	80	0
Juice of Turkish tobacco	Turkish tobacco	2	80	0
Do	do	1	20	1
Do	Beet	1	80	0
Juice of Black Valentine bean	Black Valentine bean	3	80	0
Crushed beet leaf hoppers	Beet	1	80	0
Phloem exudate from beet	do	1	124	14

[a] Numbers in this column refer to the following methods of inoculation: 1, Needle punctures into crown through drops of inoculum; 2, needle punctures into crown through diseased leaves; 3, gentle rubbing of leaves with a roll of cheesecloth saturated with inoculum; 4, inoculum injected into the hollow stem of squash or into the pith of tobacco by means of a hypodermic needle.

Büsgen (*6*), Davidson (*12*), Horsfall (*16*), Kenneth M. Smith (*25*), and others have shown that aphids, which constitute by far the most important group of vectors of plant viruses, feed on the phloem tissues of the plants on which they live. Other sucking insects feed on parenchyma or vascular tissue or both, depending on the species. Leaf hoppers as a group, obtain food material from a number of tissues. Smith and Poos (*24*) found that of 6 species studied 5 fed primarily on the mesophyll of the leaf and 1 on the phloem.

In only a few instances have careful studies been made of the feeding habits of insects in relation to transmission of plant viruses. Brandes (*5*) has shown that *Aphis maidis* Fitch, a vector of sugarcane mosaic, makes the phloem its primary objective. The stylets of this insect penetrate the epidermis directly, pass through cells and intercellular spaces of the underlying tissues, and terminate in the phloem of a vascular bundle. Kenneth M. Smith (*26*) states that the species of aphids that transmit potato leaf roll, as well as species that do not transmit this disease, feed on the phloem.

No information has been published regarding the tissues from which the beet leaf hopper obtains its food supply. Observations on leaf hoppers caged on beets indicate that they prefer the veins. This preference is especially noticeable if the leaf hoppers are feeding on petioles. In the sugar-beet petiole there are normally five or more large veins and several smaller ones arranged in an arc beneath the convex surface. The larger veins are deep-seated. The smaller veins vary in this respect, but those in the acute angles of the petioles are always very close to the surface. In feeding, leaf hoppers arrange themselves in greatest numbers along these angles as if seeking the smaller and more superficial veins.

To supplement these observations on the feeding of the beet leaf hopper, more detailed investigations have been undertaken. This work has included a microscopic study of mouth parts inserted in the tissue, and similar studies of the feeding punctures in the beet petiole by means of freehand sections of fresh petioles and by means of embedded and stained material.

In obtaining mouth parts fixed in feeding position, leaf hoppers after being starved overnight were placed on beet petioles and allowed to feed until quiet. They were then subjected to a temperature of about 28° F. for several minutes or until they became inactive. A capillary pipette filled with ether was applied to the posterior end of the abdomen of each insect that remained undisturbed on the petiole. The etherized insects were covered with melted agar to fix them firmly in place. Portions of petiole with the agar-embedded leaf hoppers were killed and fixed in Schaffner's chromo-acetic solution, and sectioned in the usual way. A very slight movement of the leaf hoppers before or during the killing and fixing processes resulted in partial or complete withdrawal of the stylets. Many leaf hoppers were found with stylets exserted from the labium but not inserted in the tissue, or only partly so. However, several leaf hoppers were satisfactorily fixed with the mouth parts apparently in normal relation to the plant tissue.

In further studies leaf hoppers were allowed to feed from 12 to 24 hours on petioles and were then removed. Some of the petioles were sectioned immediately; others were killed, embedded, sectioned, and stained. The line of puncture is quite evident in fresh material as well as in stained sections. In penetrating the tissues the leaf hoppers form a sheath which completely encases the stylets. After the stylets are withdrawn this sheath remains and a definite line through the center marks the position of the stylets. In live petioles the sheath when first laid down is almost colorless but soon takes on a yellowish coloration which clearly differentiates it from the plant tissue. In prepared sections it takes a deep safranine stain with the safranine-Delafield's haematoxylin combination (fig. 1).

A study of leaf-hopper mouth parts in feeding position in conjunction with a study of numerous punctures in fresh and prepared material furnishes a complete picture of the relation of mouth parts to the various tissues of the plant during feeding. These studies have shown clearly that the leaf hopper is able to penetrate cell walls without difficulty (figs. 2 and 3). The line of puncture extends from the epidermis through and between cell walls of the subepidermal layers, frequently to vascular bundles. The path of penetration usually is

straight and directed toward a vein. However, the path may be curved and frequently is branched near the tip (fig. 1, *A*). Apparently the stylets can be bent in only one direction at a time, but by partly withdrawing them and inserting them in another direction the leaf hopper is able to explore a considerable area. Some trails curve toward a vascular bundle from an initial direction that would have

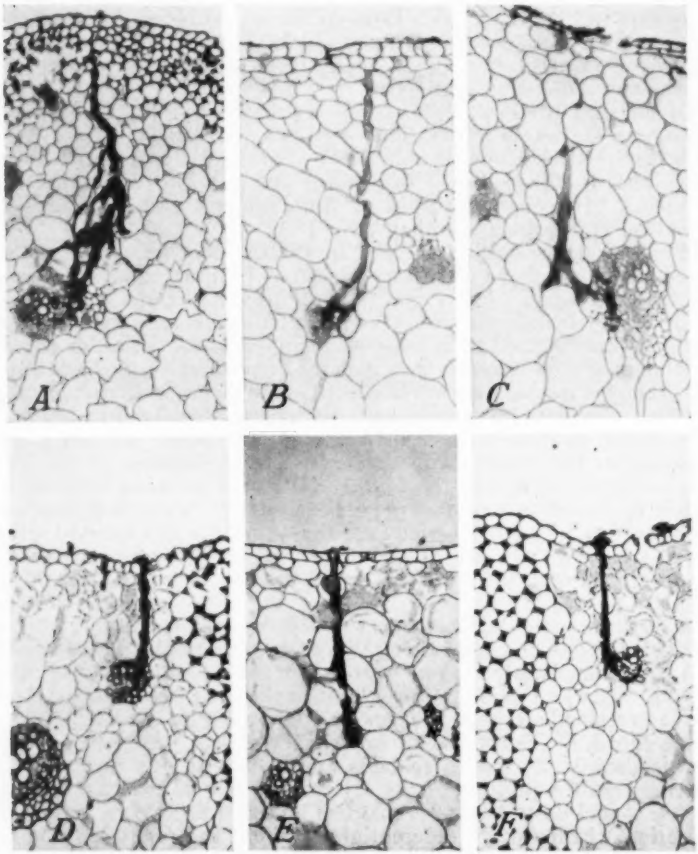

FIGURE 1, *A–F*.—Path of feeding punctures of *Eutettix tenellus* in beet petioles. Results of probing are shown in *A* and change of direction to reach vascular bundles in *B* and *C*. A puncture terminating in parenchyma is shown in *E*. × 90.

terminated in parenchyma (fig. 1, *C*). Punctures made from the xylem side of the petiole usually veer away from the middle of the bundle and enter the phloem from one side. A few instances of xylem invasion were noted in which copious quantities of exudate were deposited in the tracheae (fig. 3). Whether this happened by chance or whether leaf hoppers extract water or food from the xylem is difficult to determine. However, the number of punctures terminating in the

phloem and the amount of probing sometimes done, apparently in order to locate the phloem, indicate that this is the tissue of primary importance in supplying food.

One hundred punctures were counted and classified on the basis of the tissue in which they terminated. Of these, 24 terminated in or near the phloem of small veins; 22 terminated in or near the phloem of large veins; 46 terminated in parenchyma outside of the bundles but began from points from which bundles could have been reached.

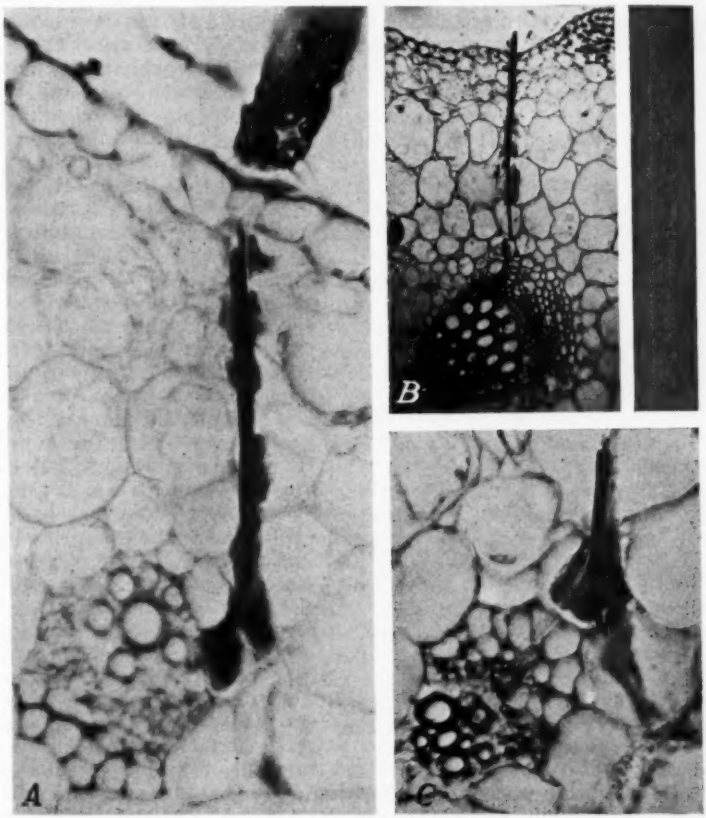

FIGURE 2.—*A-C*, Cross sections of beet petioles. The stylets of *Eutettix tenellus* are embedded in the tissue in the normal feeding position. × 90.

Only 8 trails were found in the parenchyma of the concave side of the petioles originating from points from which bundles could not have been reached. It should be stated that the section from which these counts were made came from small petioles on which large numbers of leaf hoppers had fed. Larger petioles and smaller numbers of leaf hoppers might be expected to give different results and probably would reduce the number of punctures terminating in the parenchyma outside of the bundles, many of which were very shallow and were

probably made by leaf hoppers disturbed before maximum penetration had been effected. None of the punctures in the parenchyma was branched nor was there other evidence that the leaf hopper spent much time in exploring such areas. In view of these facts and of evidence to be presented later showing that the leaf hopper derives very little of the life-sustaining materials from parenchyma, it seems probable that in making these punctures the leaf hoppers were merely searching for a more desirable medium from which to extract food.

Several investigators have mentioned the sheath material found in the feeding punctures of sucking insects, but there is a lack of agreement as to whether the sheath material is of plant or of insect origin. Büsgen (*6*) is of the opinion that the sheath laid down by the insects that he studied was composed of material excreted by the insect. Davidson (*12*) considers that the sheath wall of *Aphis rumicis* L. is "composed of substances produced by the reaction of the saliva on the cell sap." Horsfall (*16*) found that the sheath in the feeding punctures left by certain aphids contains proteid material and calcium pectate, and suggests that it is laid down by the plant cells in response to a wound stimulus though the proteid material may possibly be injected by the insect. King and Cook (*17*) suggest that the sheath produced by the sucking insects that they studied results from the action of insect saliva on the middle lamella. F. F. Smith (*23*) has shown that the sheath material in the punctures produced by the potato leaf hopper and the three-cornered alfalfa hopper is largely of insect origin and contains no plant substances with the possible exception of pectose. Brandes (*5*) states that the sheath laid down by *A. maidis* is composed of material given off by the insect. It is obvious that the sheath material formed by *Eutettix tenellus* is composed of material that is given off by the leaf hopper itself. This was demonstrated by a method similar to that described by Fife (*14*), which involved mounting a live leaf hopper under a microscope in such a position as to have the mouth parts inserted horizontally through a membrane into a liquid in the field of vision. In this position the insect may be watched in the process of forming a sheath. Many individuals begin the discharge of a colorless material as soon as the mouth parts penetrate the membrane and continue as the stylets are inserted farther into the medium. The discharge coagulates almost

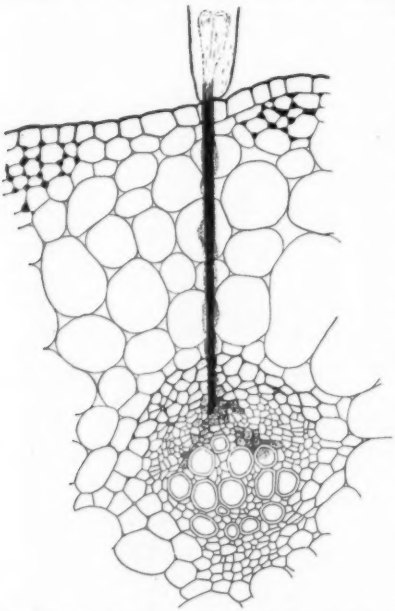

FIGURE 3.—Stylets of *Eutettix tenellus*, showing their relation to the tissues of the beet petiole during feeding. (Drawing made with the aid of a camera lucida.) ×180.

immediately and forms a distinctly visible hyaline sheath around the stylets, which in thickness and general physical properties is similar to the sheath found in freehand and stained sections of the beet petiole. Withdrawal of the stylets leaves a very definite line marking their position. With repeated penetration and partial withdrawal of the stylets a considerable mass of exudate is built up in which stylet trails extending in many directions are visible.

The materials deposited in the plant tissues by nonviruliferous leaf hoppers evidently cause very little injury to the plant as a whole, since a beet plant of average size will support a large leaf-hopper population for a considerable period with no marked ill effect.

Further investigations were made to determine the reaction of individual cells in different types of beet tissue to feeding punctures of nonviruliferous leaf hoppers. Large numbers of leaf hoppers were fed on beet petioles 24 hours and then removed. Microscopic examinations of freehand sections of these petioles were made daily for 10 days and at 5-day intervals thereafter, the last examination being made 20 days after feeding. The sheaths were at first hyaline but soon became yellowish or yellowish brown. They maintained their original relations to the cells to a remarkable degree. By the tenth day, in some instances, the sheath material was displaced in some of the cells and had shrunken slightly. It was still present and easily traced, however, on the twentieth day.

Where collenchyma was traversed, the yellowish color of the sheath was imparted to the thickened parts of the cell walls. This was true also of the cell walls of the bundle cap. Other cells in the path of punctures did not show this change in color of walls. The large parenchyma cells through which the sheaths passed reacted in different ways but all retained their turgidity for 20 days after the punctures were made. Some remained apparently normal, even retaining normal-appearing chloroplasts along with sheath material. Others had a distinctly granular protoplasmic structure and the nucleus in some cases was granular and irregular in outline. The cells were apparently very rarely dead even after 20 days. The cell-wall discoloration in the collenchyma and bundle cap had almost completely disappeared after 15 days, and the vascular bundles seemed normal except for the sheath material remaining in the cells.

Assuming that the phloem is at least the chief reservoir of virus and the place of most rapid multiplication, as stated previously by Brandes (*5*) in the case of *Aphis maidis*, it would be difficult to imagine a mechanism more perfectly designed for virus extraction and introduction than that possessed by the beet leaf hopper. The laying down of a sheath around the mouth parts as they penetrate probably effectually seals off all contents of cells external to the phloem. The introduction of sheath material into the phloem insures the introduction of salivary secretions into this tissue and probably accounts for the introduction of virus. This virus is liberated into a medium rich in nutrients and in a tissue physically adapted for the rapid distribution of inoculum to various parts of the plant, especially to the rapidly growing areas. The same insect mechanism is equally efficient in removing virus directly from the phloem without having the virus come in contact with cell contents of parenchyma tissue or with external agents.

TISSUES FROM WHICH THE LEAF HOPPER OBTAINS FOOD AND EXTRACTS VIRUS

Attempts were made to segregate certain types of beet tissue and to determine their virus content by testing the ability of leaf hoppers to obtain virus from them. These experiments have consisted chiefly of segregating parenchymatous tissues in different parts of the plant and comparing their virus content with adjacent tissues containing vascular bundles. Tissue in which there are no vacsular elements may be isolated from the ventral side of large petioles, from the crown of large beets, from the pith of the flowering stalk, and from seeds in an early stage of development.

However, before accurate conclusions regarding the virus content of tissues from these various sources may be drawn from the results of leaf-hopper transmission experiments, it is necessary to know the effect of the tissues on the leaf hopper and the relative amounts of materials that the leaf hopper is able to extract from them. To throw some light on this problem, the mortality of the leaf hoppers having access to parenchyma tissue from each of the sources just mentioned has been compared with the mortality of leaf hoppers having access to adjacent tissue containing vascular elements. The average length of life of leaf hoppers on the different tissues is taken as a rough relative measure of the food and other materials obtained.

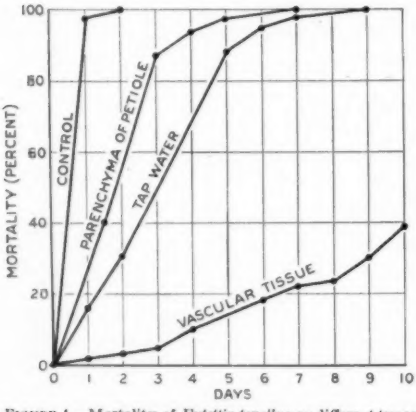

FIGURE 4.—Mortality of *Eutettix tenellus* on different types of beet tissue at 65° to 75° F.

LEAF-HOPPER MORTALITY ON DIFFERENT TYPES OF BEET TISSUE

Mortality tests were made in two separate experiments. In the first experiment, mortality of leaf hoppers having access to vascular tissue of petioles was compared with that of an equal number of leaf hoppers having access to parenchyma of the ventral side of petioles. The experiment was started with 10 petioles for each type of feeding, and 10 leaf hoppers were placed on each petiole. Fresh petioles were supplied every 48 hours. The petioles were covered with a thick coating of paraffin, with strips of paraffin about one fourth of an inch wide and 3 inches long removed to expose parenchyma tissue on the concave side in one series and vascular tissue along the acute angles of the petioles in another series. As a further check on these treatments, a third lot of 100 leaf hoppers was placed in small cages where they had access to tap water through a parchment membrane, and a fourth lot of 100 was placed in small cages without food or water. The experiment was run at relatively low temperatures (60°–75° F.) and discontinued at the end of 10 days. The results are shown graphically in figure 4.

All leaf hoppers receiving neither food nor water were dead at the end of the second day. The mortality curve of the lot receiving parenchyma is roughly parallel with that of the lot receiving tap water, although the death rate is slightly lower in the latter group; mortality reached 100 percent on the seventh and ninth days, respectively. The leaf hoppers having access to the vascular tissue thrived much better, only 39 percent being dead at the end of the tenth day.

In a second experiment, tissue from additional sources was used and the leaf hoppers were kept at a temperature of 90° to 100° F. The different lots of insects in materials available for this experiment were given, respectively, (1) neither food nor water, (2) tap water, (3) parenchyma of the petiole, (4) vascular tissue of the petiole, (5) young seeds, (6) hull of the seed ball, (7) pith from the crown and flowering stem, and (8) tissue containing vascular elements, from areas adjacent to the pith of the flowering stalk and the crown. The experiment was run in duplicate series, 50 leaf hoppers being used in each treatment in each series. The test was discontinued at the end of 48 hours. The results are shown graphically in figure 5.

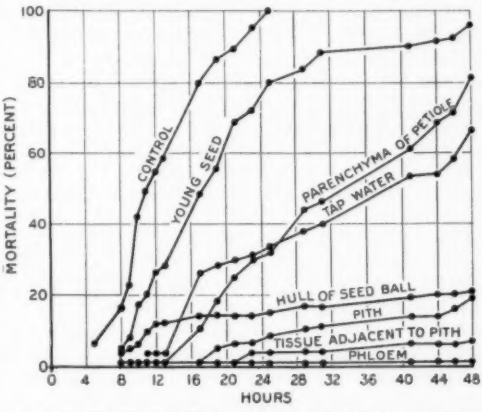

FIGURE 5.—Mortality of *Eutettix tenellus* on different types of beet tissue at 90° to 100° F.

As measured by the control treatment in which the leaf hoppers received neither food nor water, each of the types of tissue on which the insects were allowed to feed yielded some life-sustaining materials. Young seeds proved to be poorest in this respect, probably in part because of their tendency to dry very rapidly. The mortality curve, however, is steep for parenchyma from all sources except the flowering stalk and crown.

In general, these experiments indicate that parenchyma is unfavorable for the beet leaf hopper. Parenchyma of the petiole seems to be little better than tap water. Parenchyma of the flowering stalk and crown is more favorable and ranks in food value next to tissue containing vascular elements. It is worthy of note that parenchyma from the flowering stalk and crown is higher in sugar than parenchyma from other sources, and it is possible that this greater sugar content is responsible for the prolonged life of the leaf hoppers. Normally, of these types of parenchyma, only that from the petiole is available to leaf hoppers. These experiments furnish additional evidence to support the view that the beet leaf hopper is chiefly dependent on the phloem for its food.

The next question of importance is whether the leaf hopper feeds sufficiently on parenchyma from the various sources to acquire virus if it is present. Experiments have indicated that a relatively short period of feeding is sufficient for leaf hoppers to acquire virus from

diseased beet leaves. A few individuals have become viruliferous after a 1-minute feeding period, and larger numbers acquire virus as the feeding period is increased. Of 150 leaf hoppers fed singly on diseased beet leaves, 11 percent acquired the virus in 5 minutes. In another test with the same number of leaf hoppers, 23 percent became viruliferous after a 10-minute feeding. Since the evidence shows that the beet leaf hopper acquires enough material from parenchyma to appreciably prolong its life, and since the leaf hopper acquires the virus in a very short period of feeding on diseased beet leaves, it seems reasonable to assume that the leaf hopper may be used to furnish evidence regarding the presence or absence of virus in parenchymatous tissue despite the fact that parenchyma is not a very favorable source of food.

VIRUS CONTENT OF TISSUES OF BEET PLANT

Assuming that the method just described affords a means of testing for the presence of virus, nonviruliferous leaf hoppers have been given access to various types of tissue isolated from diseased beets. At the time the leaf hoppers were given access to parenchyma a second group of leaf hoppers was placed in an empty cage to serve as a check on the feeding of the leaf hoppers on parenchyma. When all the leaf hoppers serving as checks were dead the insects surviving on parenchyma were transferred to seedling plants. These checks were used in all tests except those involving the parenchyma of the petiole.

Parenchyma of Petiole

Large petioles from beets infected during the current season were covered with paraffin, and strips of this were removed to expose parenchyma or vascular tissue, as described previously. Forty nonviruliferous leaf hoppers were allowed to feed on each petiole, 20 for 24 hours on parenchyma and 20 for 24 hours on vascular tissue. One half of the petioles had parenchyma exposed during the first 24 hours and vascular elements exposed during the second 24 hours, and the other half had the exposures made in reverse order. At the end of the feeding period the leaf hoppers were divided into lots of 5 each and caged on healthy plants. In this manner 8 healthy sugar-beet plants were inoculated from each petiole; 4 plants by means of leaf hoppers which had access to parenchyma and 4 by means of leaf hoppers which had access to vascular elements. One hundred and four plants were inoculated by means of leaf hoppers from each type of tissue. Of the 104 plants inoculated by means of leaf hoppers from vascular tissue, 42 became infected; whereas, of the 104 plants inoculated by means of leaf hoppers from parenchyma, only 1 became infected. The results of this experiment are shown in table 2.

In a second experiment, petioles were taken from plants that had been diseased several months and on which petioles had been produced subsequent to infection. The plan of this experiment was the same as that just described, except that larger numbers of petioles were used and only one lot of leaf hoppers was given access to each petiole. In this test, of the 80 plants inoculated by means of leaf hoppers from tissue containing vascular elements, 37 became infected; whereas, of the 80 plants inoculated by means of leaf hoppers from parenchyma tissue, none showed any sign of disease (table 2).

TABLE 2.—*Virus content of sugar-beet tissues as indicated by leaf-hopper tests*

Tissue tested	Leaf hoppers fed	Plants inoculated [a]	Plants infected	
	Number	*Number*	*Number*	*Per cent*
Vascular tissue, petiole of first-year beets	520	104	42	40.3
Parenchyma tissue, petiole of first-year beets	520	104	1	.9
Vascular tissue, petiole of second-year beets	400	80	37	46.2
Parenchyma tissue, petiole of second-year beets	400	80	0	.0
Vascular tissue, flowering stalk	420	84	62	73.8
Pith of flowering stalk	420	84	0	.0
Vascular tissue below crown	600	120	58	48.3
Parenchyma tissue below crown	600	120	9	7.5
Outer hull of seed ball	200	40	11	27.5
Young seeds	200	40	0	.0

[a] 5 leaf hoppers were placed on each plant inoculated.

PARENCHYMA OF CROWN

Tissue selected as containing no vascular elements was taken from the crown of large diseased beets and placed in a cage containing nonviruliferous leaf hoppers that had been starved overnight. A second lot of tissue containing vascular elements was taken from the portion of the beet adjacent to the first selection and exposed to a second lot of leaf hoppers. After the leaf hoppers had been allowed a feeding period of 5 hours they were divided into lots of 5 each and placed on healthy beet seedlings. One hundred and twenty plants were inoculated by means of leaf hoppers from each of the two food sources. In this experiment, of the 120 plants inoculated by means of leaf hoppers from tissue containing vascular elements, 58 became infected; and of the 120 plants inoculated by means of leaf hoppers from tissue containing no vascular elements, 9 became infected.

In the foregoing experiment, tissue was taken from six beets and the tissue from each beet was used as a separate test. For the six beets the number of infections resulting from the leaf hoppers that had access to parenchyma tissue was, respectively, 0, 1, 5, 0, 3, and 0. The infections resulting from leaf hoppers that had fed on adjacent vascular tissue from the same beets were, respectively, 6, 9, 18, 16, 9, and 5. Each value represents the number of infections obtained from inoculating 20 plants.

These results seem to demonstrate that virus does occur in some types of parenchyma tissue. Perhaps if virus occurs in any parenchyma tissue of the plant it would be expected to be present in the parenchyma that lies immediately below the growing point and closest to the actively growing areas of the beet crown. However, even there it seems to occur in diminished concentrations—in some cases in concentrations too low for relatively large numbers of leaf hoppers to pick it up.

PITH OF FLOWERING STALK

Large fruiting stalks were selected from plants that had been infected the season prior to flowering. Portions of pith containing no vascular tissue were removed and exposed to the feeding of nonviruliferous leaf hoppers. A second group of nonviruliferous leaf hoppers was allowed to feed on tissue containing vascular elements selected from the area immediately adjacent to the pith that was used as food for the first lot. Eighty-four plants were inoculated from each lot of leaf hoppers. Of the 84 plants inoculated by means of the leaf

hoppers from tissue containing vascular elements, 62 became infected; whereas of the 84 plants inoculated by means of the leaf hoppers from pith, none showed any sign of disease.

Young Seeds

Severin (*21*) has shown that there is no seed transmission of the curly-top virus in beet. Since this is true, the question arises as to whether the virus never gains access to the seed in any stage of its development or whether it may be present in certain early stages of seed development and become inactivated as the seed matures.

To obtain information as to whether virus occurs in seeds in the earlier stages of their development, young seeds from diseased plants were separated from the surrounding tissue and placed in cages where nonviruliferous leaf hoppers had access to them. Very young seeds having a high water content were selected. As a check on the virus content of the nearby tissue, the hulls of the seed balls from which the seeds were removed were placed in cages with other nonviruliferous leaf hoppers. After periods of several hours the leaf hoppers were divided into groups of five each and placed on seedling beets. Of the 40 plants inoculated by means of leaf hoppers from hulls, 11 showed signs of the disease; whereas of the 40 plants inoculated by means of leaf hoppers from seeds, none became infected.

Since no virus was obtained from the seeds by the leaf hoppers, it seems probable that seeds contain no virus even in the early stages of their development and that virus may not be able to pass from the plant into the seed. Therefore, absence of seed transmission may be due to a barrier between the embryo and the mother plant which, although permitting passage of water, mineral elements, and elaborated foods, restrains or inactivates the virus.

CONCENTRATION OF VIRUS IN PHLOEM EXUDATE

The foregoing experiments show that leaf hoppers readily acquire virus from vascular tissue and rarely obtain it from other tissues. Since the xylem elements evidently do not carry any considerable amount of virus, the phloem must contain at least the greater part of the virus in the vascular elements. Liquid from phloem tissue may be obtained by making cuts across the tops of beet roots. In a few minutes drops of exudate appear above the severed ends of vascular bundles and may be collected with capillary tubes and used in artificial feeding tests with leaf hoppers. Attempts were made to compare the relative virus concentration of such exudate with that of the expressed juice from the entire beet. In these tests, drops of exudate from diseased beets were placed on a parchment membrane. Nonviruliferous leaf hoppers were allowed to feed on the exudate through the membrane for about 4 hours and then were caged singly on seedling beets.

A second lot of nonviruliferous leaf hoppers were allowed to feed on expressed beet juice and then were caged singly on seedling beets. The results shown in table 3 indicate that more virus is available to the leaf hopper from phloem exudate than is available from expressed juice from the entire beet. Of the 104 leaf hoppers fed on phloem exudate, 33 produced infection; whereas of the 104 leaf hoppers having access to expressed beet juice, only 4 gave evidence of having acquired virus.

TABLE 3.—*Virus content of phloem exudate and expressed beet juice as indicated by leaf-hopper tests*

Material tested	Plants inoculated [a]	Plants infected	
	Number	*Number*	*Percent*
Exudate from phloem of beet root	104	33	31.7
Expressed juice from beet root	104	4	3.8
Exudate from beet petioles	24	7	29.1
Expressed juice from beet petioles	24	0	.0

[a] 1 leaf hopper was placed on each plant inoculated.

It is noted frequently that drops of exudate collect on the leaves and petioles of rapidly growing beets that have been recently infected. This exudate has long been considered to come from the phloem. Recently, Esau (*13*) has made histological studies of diseased beets and described the path which this exudate takes in moving from the phloem to the exterior. By means of exudate of this type further feeding tests were made to determine virus concentration. These tests were carried out as already described, 24 leaf hoppers being given access to exudate and an equal number having access to expressed juice from beet petioles. In this test, 7 leaf hoppers acquired the virus from exudate, whereas none was found to be viruliferous after feeding on expressed juice.

In connection with the experiments that indicate a very low concentration of virus in types of tissue other than phloem, these tests with phloem exudate seem to demonstrate conclusively that the chief virus reservoir in the sugar beet is phloem tissue. Phloem exudate with its high virus content may prove valuable in virus purification experiments and in work dealing with properties of the virus.

MOVEMENT OF VIRUS IN DIFFERENT TISSUES

GRAFT UNIONS OF SUGAR BEET

Twenty healthy sugar-beet plants having main roots approximately three fourths of an inch in diameter were taken from the soil, and a portion of one side of each root was removed by means of a sharp knife. Twenty diseased beet plants were treated in a similar manner. The cut surface of each diseased beet was placed in contact with the cut surface of a healthy beet and the two plants firmly bound together and potted. Symptoms of curly top began to appear on the new leaves of the inoculated beets in 3 weeks. Of the 20 healthy beets, 17 became diseased. In the case of the 3 beets that did not develop symptoms, the diseased member of the pair died probably before union was complete.

GRAFT UNIONS OF TOBACCO

Experiments were conducted with Turkish tobacco to determine at what stage in the development of a graft union the curly-top virus passes from a diseased scion to a healthy stock. Healthy tobacco plants were cut back to a height of about 8 inches, and 3 inches of stem from a diseased plant was grafted to each healthy plant. Cuts were made at angles that afforded considerable surface contact. Grafts were removed from stocks at 24-hour intervals for 15 days and the results on the inoculated plants noted (table 4).

TABLE 4.—*Time required for infection of healthy tobacco stocks by scions from diseased plants*

Period between grafting and removal of diseased scion (days)	Plants grafted	Plants infected		Period between grafting and removal of diseased scion (days)	Plants grafted	Plants infected	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
1	10	0	0	9	12	7	58
2	10	0	0	10	10	7	70
3	10	0	0	11	10	8	80
4	10	0	0	12	10	10	100
5	10	0	0	13	10	9	90
6	13	0	0	14	10	10	100
7	11	3	27	15	10	9	90
8	12	5	41				

No infection occurred until the seventh day. The number of infected plants increased from 27 percent on the seventh day to 100 percent on the twelfth day. The union between the stock and the scion was examined for all the time intervals used. At the end of 3 days a definite union was found which was complete enough to make necessary the use of an appreciable amount of force in removing the scion. Graft unions of different ages from 3 to 9 days were killed, embedded, and sectioned, and then were examined under a compound microscope. In all the specimens sectioned, the union at the end of the third day was composed wholly of meristematic tissue. This rapidly differentiated into other tissues and in the 5- and 6-day-old unions the beginnings of tracheal elements were clearly evident. In the 7-, 8-, and 9-day-old unions, apparently mature tracheal elements with pits and rings were distinctly visible. The walls of these elements were lignified, as indicated by their taking a deep safranine stain. Phloem elements could not be clearly identified, but strands of elongated cells paralleling the tracheae were observed, which may well have included functional phloem.

The foregoing experiment seems to demonstrate that in tobacco infection does not result from contact of cut surfaces and that virus does not move through newly formed meristem in a period of from 2 to 4 days. Infection apparently does not occur until after tracheal elements, and probably phloem elements, connect the stock with the scion. Since the virus seems unable to pass out of tracheae into other cells and become established sufficiently to produce disease, and since it also seems unable to pass through meristem or young parenchyma, it appears extremely probable that in tobacco grafts the virus moves in the phloem in crossing a graft union.

REMOVAL OF RINGS OF BARK[3] AND INTERNAL PHLOEM

Killing portions of stems and removing rings of bark have been the methods used by several investigators to obtain evidence regarding the tissues in which virus is dispersed through plants.

The writer (*2, 3*) found that the virus causing curl of raspberry and two viruses causing mosaic of raspberry may be limited in their movement through the plant by removing rings of bark.

In tomato, according to Caldwell (*7*), the virus of mosaic bridged an area on the stem from which a ring of bark was removed. As

[3] The term "bark" as used in this paper signifies all tissue of the stem from epidermis to cambium, inclusive.

Caldwell points out, however, the tomato has a weak development of internal phloem, so that it is not clear whether the virus passed through the woody cylinder, the pith, or the internal phloem. The same investigator conducted experiments to determine whether the virus moved through dead portions of stem. Of the 26 plants having portions of the stems treated with chloroform, the virus in 14 did not cross the treated area. In 12 plants, virus was present in noninoculated parts. These exceptions are attributed to incomplete killing of the stem, regeneration of tissue, and accidental infection. In plants having portions of the stems killed by steaming, the virus was held in the inoculated parts for several weeks in 20 of the 21 plants used. The one exception was thought to be due to accidental infection and not to movement of virus across the steamed area.

The virus causing curly top has certain characteristics that render it convenient for experimental use in tests involving tissues through which movement occurs. One of the most important of these is its failure to produce infection except as inoculated into plants by its specific vector, thus reducing to a minimum the chance infection of noninoculated parts of plants kept for long periods of time. Moreover, the virus causes disease in a large number of plant species, thus making available a wide range of anatomical types for experimental use.

Since the sugar beet cannot be employed in ringing experiments because of its anatomical structure, it was necessary to choose some other susceptible plant for such experiments. In making a survey of susceptible plants of a more or less woody nature, tests were made on two species of tobacco, namely, common tobacco (*Nicotiana tabacum* L.), Turkish variety, and tree tobacco (*N. glauca* Graham). These plants grow well under a wide range of greenhouse conditions, are easily propagated at all seasons from seeds or cuttings, and are very satisfactory for experiments involving grafting. Moreover, the presence of an internal phloem makes these species suitable for experiments on the movement of virus in the phloem not possible in most other types of woody plants. Each of these species has been used in a fairly extensive series of ringing experiments.

Nicotiana Tabacum

Turkish tobacco has only a medium degree of susceptibility to infection by the curly-top virus, but symptoms of disease are characteristic and well marked. Infection may be induced by leaf hoppers or by grafting. In most of the experiments about to be described leaf hoppers were used in making inoculations.

The first experiments with Turkish tobacco were planned to determine whether the virus would pass downward through a killed portion of stem. Plants approximately 2 feet tall were cut back to a height of about 10 inches and the buds in the leaf axils allowed to grow to a length of 1 to 2 inches. A portion of the stem below the second or third bud from the top was incased in a celluloid cylinder, the bottom being closed around the stem. Hot paraffin was poured into the cylinder. This killed the tissues and protected and supported the killed area. After a portion of the stem had been killed in this manner, leaf hoppers were allowed to feed on the top bud. Distinct

symptoms of curly top appeared in an average period of 7 days on all the inoculated buds of the 10 plants used. The inoculated parts lived for an average period of 16 days. All plants were held for

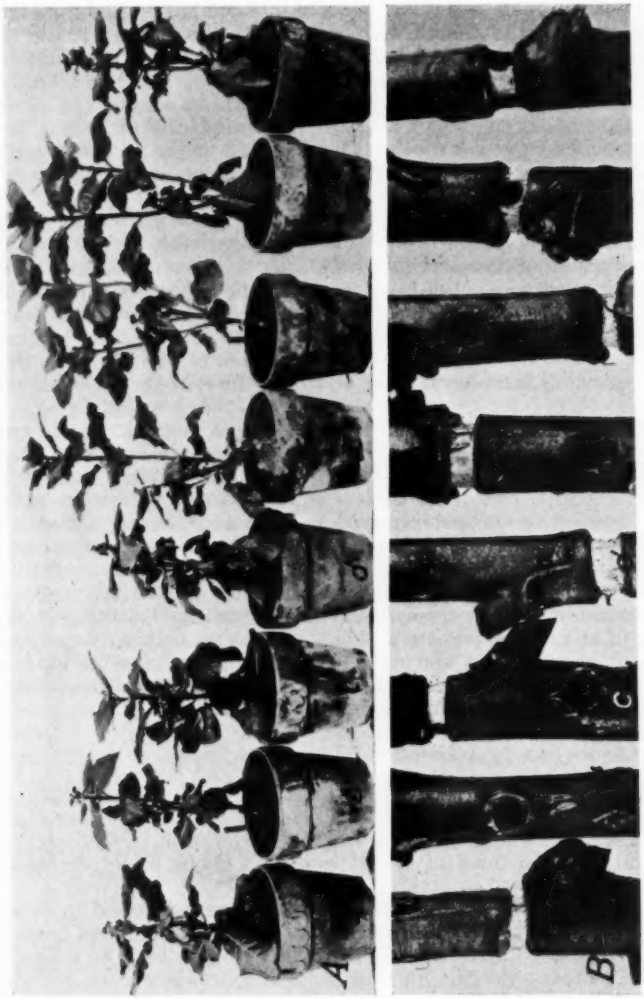

FIGURE 6.—*Nicotiana tabacum* plants after ringing. *A*, Series of plants showing results of interference with virus movement by certain types of rings. The virus moved past the rings in plants *a*, *b*, *c*, *d*, and *h*, and failed to move past the rings in plants *e*, *f*, and *g*, as shown by symptoms and lack of symptoms, respectively, on the shoots coming from below the rings. *B a–h*, Ringed areas of plants shown in *A*. (Plants photographed 1 month after inoculation.)

several weeks, but curly-top symptoms appeared in no case in any part of the plant below the killed area.

Further experiments were made to determine the part of the stem through which the virus is able to move. Plants were grown to the beginning of flower-bud production. The stems were pruned back

to a height of 18 inches and buds were allowed to start. The plants were then divided into 8 lots and treated as follows:

LOT 1.—A ring of bark was removed from the internode below the second or third bud from the top. This is termed an external ring (fig. 6, *B*, *a*).

LOT 2.—A hole was made through the bark and wood of the internode about one half of an inch below the second or third bud, by means of a small cork borer and the pith and internal phloem were removed, exposing a ring of wood one fourth of an inch wide on the inner side of the woody cylinder. This is termed an internal ring (fig. 6, *B*, *b*).

LOT 3.—An outer and inner ring 1 inch apart were made with the outer ring above the inner and the two rings separated by the second or third bud from the top of the plant (fig. 6, *B*, *c*).

LOT 4.—The plants were treated as in lot 3, except that the relative positions of the two rings were reversed (fig. 6, *B*, *d*).

LOT 5.—An outer and an inner ring, 1 inch apart, were made with the outer ring above the inner and both rings in the second or third internode (fig. 6, *B*, *e*).

LOT 6.—The plants were treated as in lot 5 except that the relative positions of the rings were reversed (fig. 6, *B*, *f*).

LOT 7.—Outer and inner rings were made in the second or third internode, the two rings being placed at the same level on the stem (fig. 6, *B*, *g*).

LOT 8.—Plants were treated as in lot 7, except that a small strand of bark was left in the outer ring (fig. 6, *B*, *h*).

These treatments were designed to determine whether the curly-top virus moves (1) through internal phloem, (2) through the external phloem, (3) from the internal phloem to the external phloem through unions of the two in the leaf traces, (4) from the external to the internal phloem through unions of the two in the leaf traces, (5) from the internal phloem to the external phloem through the medullary rays or other parts of the woody cylinder, (6) from the external phloem to the internal phloem through the medullary rays or other parts of the woody cylinder, (7) downward through the woody cylinder, or (8) through a very small strand of bark bridging an external ring.

Immediately after ringing, the top bud on each plant was exposed to viruliferous leaf hoppers. Symptoms of curly top appeared on the inoculated bud in from 6 to 13 days. Typical results of a series of tests are shown in figure 6, *A*, and results of all tests are given in table 5.

TABLE 5.—*Influence of rings on virus movement in plants of Turkish tobacco (Nicotiana tabacum) cut back to a height of 18 inches, inoculated in top two buds, and ringed 1 to 3 inches below the point of inoculation*

Number and position of rings	Plants infected	Effect on plants in which virus passed rings		Effect on plants in which virus did not pass rings	
		Plants affected	Average period between inoculation and appearance of symptoms	Plants affected	Average period between inoculation and death of parts above rings
	Number	*Number*	*Days*	*Number*	*Days*
Outer ring only	15	15	9.9	0	
Inner ring only	8	8	11.0	0	
Outer ring 1 inch above inner, bud between	15	15	9.6	0	
Outer ring 1 inch below inner, bud between	15	15	11.1	0	
Outer ring 1 inch above inner, both in internode	11	4	35.0	7	83.8
Outer ring 1 inch below inner, both in internode	16	3	27.0	13	97.5
Outer and inner rings at same level	14	0		14	86.9
Outer and inner rings at same level, strip of bark in outer ring	7	7	10.4	0	

In all cases where there was an uninterrupted channel through either internal or external phloem across the rings the virus moved downward with little apparent delay. In many instances in some types of ringing, symptoms were evident on shoots below the rings before any sign of disease appeared on the inoculated parts. In the type of ringing in which the appearance of virus below the ring was dependent on its movement through a distance of less than one fourth of an inch of woody cylinder, no symptoms appeared in any parts below the rings in any of the 14 plants inoculated. The diseased parts above the rings lived for an average period of 86.9 days from the time of inocu-

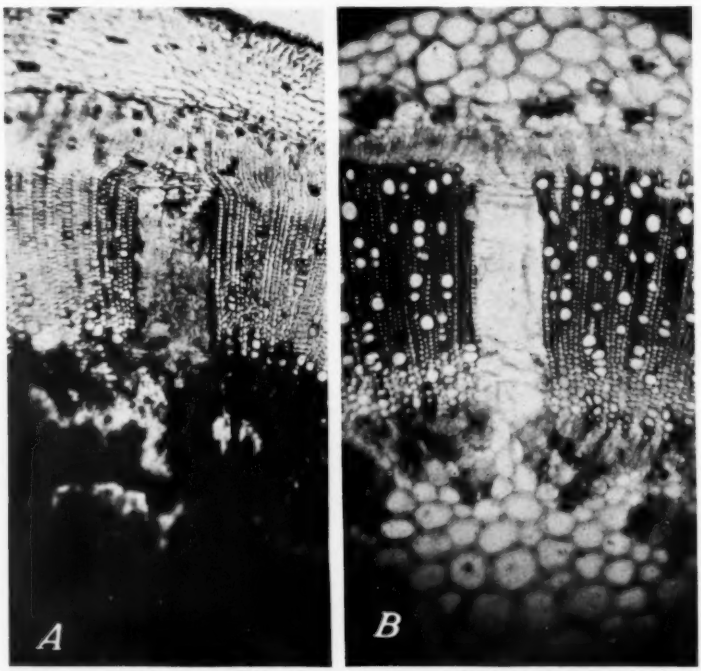

FIGURE 7.—Regeneration of cells in the woody cylinder of tobacco plants having two rings 1 inch apart in an internode: *A*, *Nicotiana tabacum*; *B*, *N. glauca*. × 40.

lation. In the series of tests in which the two rings were at different levels in the internode and the external ring was the higher, the virus was held in the inoculated parts in 7 of the 11 plants used. The inoculated parts in these 7 plants lived an average period of 83.8 days. In four plants symptoms appeared below the rings in an average period of 35 days. In the series in which both rings were at different levels in an internode and the internal ring was the higher, the virus was held in the inoculated parts in 13 of the 16 plants used, the average life of tops of the 13 plants being 97.5 days. In three plants symptoms appeared in parts below the rings in an average period of 27 days. In series in which there was an uninterrupted path of phloem across the rings, symptoms appeared on the parts below rings in an average period of 9.6 to 11.1 days, which differed in different series.

The seven plants in which symptoms appeared below rings in the two series in which the rings were at different levels in an internode were examined to determine what conditions were present or what changes had occurred that might have a bearing on an explanation of virus passage. Serial sections of entire internodes revealed definite regions of regeneration of what appeared to be medullary rays in one or more areas of the woody cylinder. These regions were sufficiently evident to be recognizable to the unaided eye and seemed to consist of masses of newly formed tissue extending from a region of very active internal phloem outward and downward to the bark, splitting the wood in their growth. These interphloem strands seemed to have their origin just above the top of the internal ring. It is considered probable that the internal phloem transports considerable food under normal conditions but that since the internal phloem is enclosed by a rigid tissue the opportunity for the expression of growth impulses may be limited, and the tendency to initiate regeneration of medullary rays may be greater than from the opposite side of the woody cylinder, where the food brought down in the external phloem is used to form a great amount of callus, wood, bark, and roots. A typical regeneration area is shown in figure 7, *A*. These areas of regenerated tissue were examined microscopically for phloem elements. They were found to be composed of a large variety of active cells of different shapes and sizes, some narrow and very much elongated, others broader but rectangular, and many oval or irregular. Phloem tissue could not be identified definitely, but it seems quite probable under the circumstances that tissue capable of translocating elaborated foods was present in these connecting strands.

Nicotiana Glauca

To have available a more woody stem, a perennial species of tobacco, *Nicotiana glauca*, was chosen for several series of ringing experiments similar to those described for *N. tabacum*. *N. glauca*, however, has not been reported as susceptible to curly top, and before the ringing experiments were started preliminary tests were made to determine the reaction of the species to the curly-top virus. To test susceptibility to infection, large numbers of viruliferous leaf hoppers were allowed to feed on five small plants for several days. None of these plants developed symptoms of curly top. Larger plants, 3 feet or more in height, were then used. These were pruned to a height of about 18 inches, and a 3-inch length of stem from a plant of *N. tabacum* affected with curly top was grafted at the top. The *N. tabacum* scions grew well and developed typical curly-top symptoms. No signs of disease appeared, however, on any of the new growth from the stock. Parts of stems from healthy *N. tabacum* plants were then grafted in at the base of the *N. glauca* stems at points about 14 inches below the diseased scion. These grafts without exception became diseased, showing that the *N. glauca* plants were infected and that the virus had moved downward through approximately 14 inches of stem.

It was later proved that *Nicotiana glauca* is susceptible to infection by direct feeding of leaf hoppers. Healthy *N. tabacum* stems were grafted into the base of *N. glauca* plants and viruliferous leaf hoppers were allowed to feed on young shoots of the *N. glauca* stems. The virus passed from the inoculated shoots through the stem and infected the graft several inches below.

Tests were next made to determine how long the virus would remain active in *Nicotiana glauca*. Two plants which had been inoculated from *N. tabacum* by the grafting method were selected and all *N. tabacum* tissue removed. These two plants were tested at intervals over a long period by grafting portions of their stems on healthy *N. tabacum* plants. The production of disease in this latter species showed that the virus was active in *N. glauca* 2 years after the plants were infected. At no time, however, were signs of curly top visible on these plants. The species seems to be a symptomless carrier of the virus under the conditions of these tests.

The stem of *Nicotiana glauca* is very well adapted to ringing experiments, but since the plant does not show symptoms of the presence of virus it was necessary to modify the technic used in previous experiments with *N. tabacum*. A suitable modification was accomplished by grafting *N. tabacum* on *N. glauca* stems as follows: *N. glauca* plants were grown in 12-inch pots to a height of 3 to 7 feet and pruned to a height of 18 inches. Plants were inoculated by grafting infected *N. tabacum* stems at the top. A second portion of stems from *N. tabacum*, this part from a healthy plant, was grafted in at the base about 14 inches below the top or diseased graft. This latter graft was used as an indicator of the presence of virus in the basal portions of the *N. glauca* plants, and the *N. glauca* stem was used as a medium in which to study the influence of rings in the downward movement of virus.

The rings were placed on the *Nicotiana glauca* stems about 1 inch below the point of union with the upper graft of *N. tabacum*. The rings were made as described for *N. tabacum* with the additions shown in table 6, where the results of this experiment are tabulated.

TABLE 6.—*Influence of rings on virus movement in Nicotiana glauca plants cut back to a height of 18 inches and grafted to curly-top Turkish tobacco at top and to healthy Turkish tobacco at base*

Number and position of rings	Plants inoculated	Effect on plants in which virus passed rings		Effect on plants in which virus did not pass rings	
		Plants affected	Average period between inoculation and appearance of symptoms below rings	Plants affected	Average period between inoculation and death of part above rings
	Number	*Number*	*Days*	*Number*	*Days*
Neither inner nor outer rings	10	10	23.6		
Outer ring only	15	15	23.8		
Inner ring only	15	15	22.7		
Outer ring 1 inch above inner, bud between	15	15	25.5		
Outer ring 1 inch below inner, bud between	15	15	22.5		
Outer ring 1 inch above inner, both in an internode	15	3	63.0	12	245
Outer ring 1 inch below inner, both in an internode	15	1	33.0	14	261
Outer and inner rings at same level in an internode	15	0		15	203
Outer and inner rings at same level, strip of bark in outer ring	10	10	19.5		
Outer and inner rings at same level, strip of phloem in inner ring	5	5	26.2		
Outer ring 1 inch above inner, both in internode, strip of bark in outer ring	6	6	19.6		
Outer ring 1 inch below inner, both in internode, strip of bark in outer ring	5	5	20.0		

Plants having no rings required about the same average length of time for the appearance of symptoms on the lower graft as those in the groups having only an external or an internal ring. Two rings 1 inch apart seemed to have no influence in delaying the passage of virus in plants where the two rings were separated by a bud, regardless of their relative positions. The average length of time required for the appearance of symptoms was 25.5 days in plants where the outer ring was above the bud and 22.5 days in plants where the inner ring was above the bud. Whether this difference of 3 days is significant may be questioned. However, it is worthy of note that in the plants requiring the longer period for the appearance of symptoms on the lower graft the rings were so placed that materials moving downward in the phloem would be required to pass outward to the external phloem from the internal phloem through connection in the leaf traces.

Two rings, one external and the other internal, 1 inch apart, and both in an internode, prevented the passage of virus in 26 of 30 plants in the two series shown in table 6. In these two series, the 4 plants in which the virus passed the rings were of the same age and had been inoculated and ringed at the same time. At the time of inoculation they had relatively immature stems with thin woody cylinders. Serial sections of the internodes in which the two rings were located in all 4 plants revealed strands of newly formed tissue extending from the internal phloem at the top of the internal ring downward to the external phloem of the bark. Figure 7, *B*, shows a section of one of these strands.

In 15 plants having two rings at the same level in an internode, there was no instance in which the virus passed the rings. The average length of life of the diseased parts above the rings was 203 days. Figure 8, *A*, shows a plant of this series that retained virus above the ring for more than a year with no movement across the ring during this time.

The presence of a small strand of either internal or external phloem bridging rings which otherwise prevented virus passage, permitted the virus to pass with no measurable delay. The influence of a small strand of bark as compared with complete severing of phloem continuity is illustrated in figure 9, *A* and *B*. The influence of a bud between the internal and external rings is illustrated in figure 10, *A* and *B*.

RATE OF VIRUS MOVEMENT

A better understanding of the movement of virus in plants and of the factors influencing it would throw new light on some of the fundamental problems presented by plant viruses. The subject of virus movement in plants has received attention from several investigators. Sufficient evidence has been accumulated to indicate considerable variation in the behavior of different viruses. Whether this variation is due to the specific nature of the viruses or to the functioning of the plants in which they occur is a question of considerable interest and importance.

McCubbin and Smith (*18*) were among the first to present data on the rate of dispersion of viruses in plants. They measured the rate of movement of the virus of tomato mosaic by layering tomato plants, inoculating them at the distal end of one of the branches, and severing the stems between the rooted portions at different distances from the

point of inoculation at known time intervals. By this means they were able to calculate a rate of movement of 1 to 2 inches per day or 1 to 2 mm per hour.

Severin (*22*) inoculated the distal ends of beet leaves with the curly-top virus by means of leaf hoppers and severed the inoculated

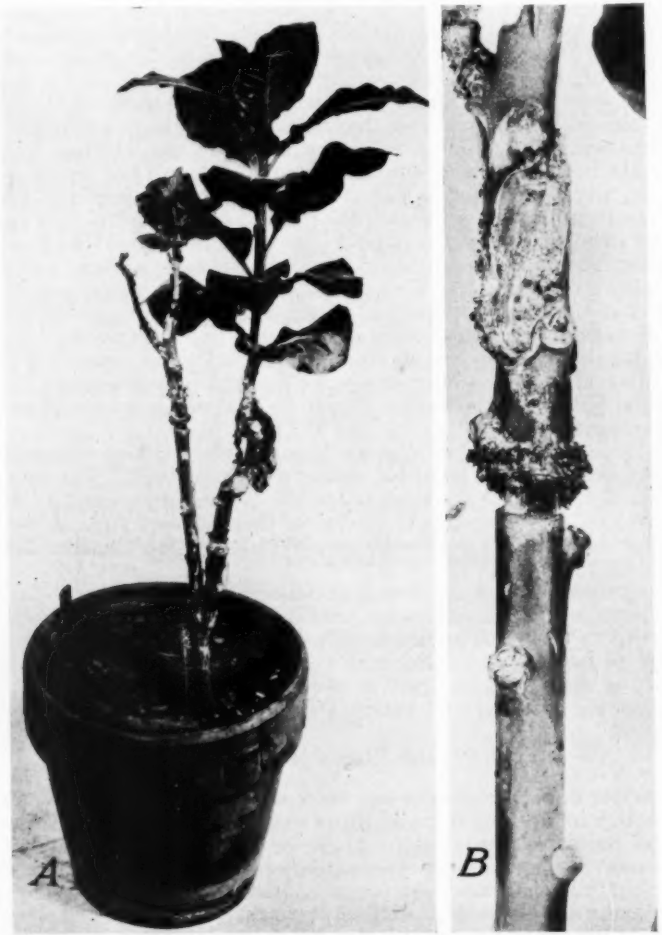

FIGURE 8.—*A*, *Nicotiana glauca* plant that had a diseased *N. tabacum* graft placed at the top and a healthy *N. tabacum* graft placed at the base and rings of internal and external phloem removed at the same level immediately below the top graft. After 420 days, virus was present in the part above the rings and absent in the parts below the rings. *B*, Ringed area, natural size. (Photographed 1 year after grafting.)

leaves at measured distances from the point of inoculation after different time intervals. The most rapid movement measured by this means was 7 inches in 30 minutes, or a rate of 14 inches per hour.

Storey (*27*), using methods similar to those of Severin, found that in 3 of 8 plants the virus of maize streak moved downward from the

point of inoculation at the distal end of a maize leaf, a distance of 40 cm, in 2 hours.

Böning (*4*) found that the virus of tobacco mosaic moved 13 cm in 2 days in tobacco and 9 cm in 2 days in tomato.

Holmes (*15*), although not attempting an accurate measurement of maximum virus movement, has presented some very interesting studies on rate of invasion of tobacco plants by the mosaic virus.

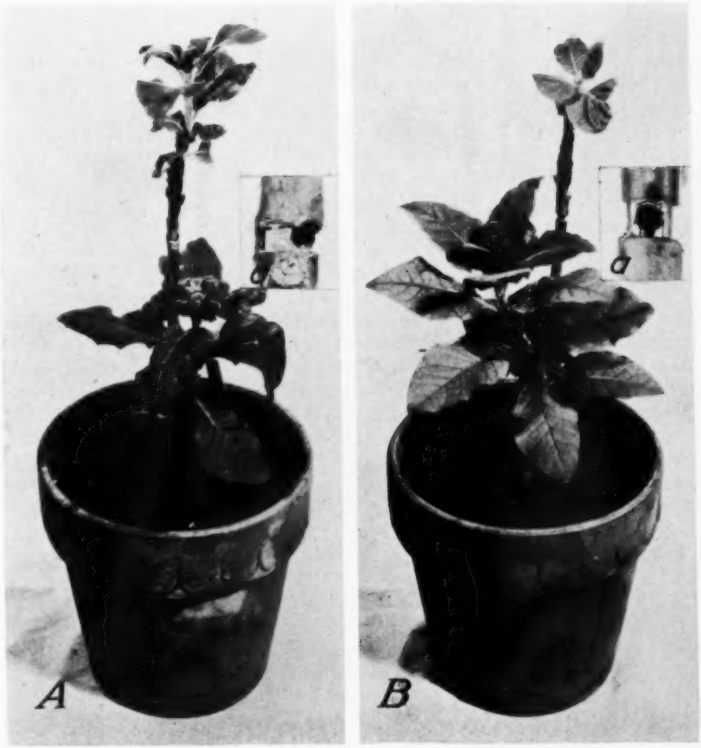

FIGURE 9.—Effect of a small strand of bark on the downward movement of virus past rings in *Nicotiana glauca*: *A*, Plant having internal and external rings at same level and a strip of bark in the outer ring. Curly-top symptoms may be seen on the lower graft. *B*, Plant having rings as in *A* but no strip of bark in the outer ring. The lower graft shows no curly-top symptoms. *A*, *a*, and *B*, *a*, Rings of respective plants, natural size. (Photographed 40 days after inoculation.)

He shows that the rate is at first very slow until the vascular bundle is reached. The virus moves more rapidly along the veins traversing the leaf blade and petiole. Apparently, the speed of movement is again accelerated when the virus passes into the stem from the inoculated leaf.

These results indicate a wide range in rate of movement among viruses in affected plants. This range extends from a rate of 1 to 2 inches per day in the tomato mosaic virus to a rate of 14 inches per hour in the virus of curly top. The factors determining these wide differences are of interest. Among the factors that may exert an

influence at the time of testing are the specific nature of the tissue inoculated, the environmental conditions, the physiologic tone of the plant, and the species or variety of the plant. An effort has been made to extend the work done by Severin and to make further

FIGURE 10.—Effect of a bud between internal and external rings on the passage of virus downward in a stem of *Nicotiana glauca: A*, Plant having external and internal rings and bud between. Note symptoms on the lower graft. *B*, Plant having internal and external rings in an internode. Note the absence of symptoms on the lower graft. (Photographed 40 days after inoculation.)

determinations on the rate of movement of the curly-top virus. Tobacco and sugar beet were used in these experiments.

TOBACCO

Measurements were made of the rate of the downward movement of virus in the stems of the Turkish variety of *Nicotiana tabacum*. All the plants were more than 24 inches high at the time of their selection for this experiment, and some of them were showing the first

indication of blossom buds. The youngest leaves of each plant were enclosed in a celluloid cage into which 50 to 100 viruliferous leaf hoppers were placed. The leaf hoppers were allowed to feed 5 hours.

The inoculated plants were incubated for different periods of time. At the end of the period allowed for virus movement they were cut off at a point 24 inches below the lowest point of inoculation, and all leaves were removed except the small ones on which the leaf hoppers fed. The stem was next cut into eight parts, each 3 inches long, and the segments placed in sand. In nearly all cases these cuttings rooted readily and produced a very satisfactory growth. The appearance of curly-top symptoms on a cutting indicated that the virus had reached that particular part of the stem in the period allowed for downward movement. The results of this experiment are shown in table 7. The virus did not move out of the inoculated 3 inches of the stem in any plant in 24 hours. In 48 hours the virus moved a distance of 24 inches in plant 10, or at a rate of one half inch per hour. The extent of stem invasion increased irregularly with the period allowed for virus movement up to 144 hours, when the virus had in all cases moved through the full 24 inches of stem and had reached the root of the plant. As calculated from these data, the maximum rate of virus movement is one half inch per hour (plant 10) for a 48-hour period.

Table 7 shows that there were decided differences among individual plants. For example, in plants 16 and 19 the virus had not moved out of the inoculated 3 inches in 96 hours, whereas in plant 17 it moved 24 inches in the same length of time. Perhaps the most interesting results were obtained from plant 20, in the 96-hour incubation period and from plants 23 and 24, in the 120-hour period. In plant 20 sections 1, 2, 3, 5, and 6 and the root portion were diseased and sections 4, 7, and 8 were healthy. In plant 23 sections 7 and 8 were diseased, whereas all the other sections were healthy, including the inoculated tip and the root below the 24-inch mark. In plant 24 sections 4, 5, and 7 were healthy and all the other sections were diseased. In all these plants, as usual in the experiment, the sections recorded as diseased showed symptoms on the first leaves from the buds and continued to show marked symptoms so long as they lived or until they were discarded. All the cuttings from these three plants were transferred to soil in 8-inch pots and the healthy-appearing ones were grown to flowering. The plants were then further tested for the presence of virus by grafting portions of the stems on healthy plants. In no case was evidence of the presence of virus obtained in plants grown from sections which had shown no symptoms. Symptoms were present at all times in all plants grown from other sections.

The significance of this erratic distribution of virus is not clear. In plants 20 and 24 it might well be that in its movement downward the virus failed to come into contact with tissue in segments 4, 7, and 8, and in segments 4, 5, and 7, respectively, in which it could become established and multiply. In plant 23, however, it is diffcult to account for the absence of virus in the inoculated portion and its presence in two segments farther down the stem.

TABLE 7.—*Rate of downward movement of curly-top virus in stems of Turkish tobacco (Nicotiana tabacum)*

Plant no.	Period between inoculating and making cuttings	Infection [a] of indicated 3-inch cutting [b] and root								
		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Root
	Hours									
1	24	+	−	−	−	−	−	−	−	−
2	24	+	−	−	−	−	−	−	−	−
3	24	+	−	−	−	−	−	−	−	−
4	24	+	−	−	−	−	−	−	−	−
5	24	+	−	−	−	−	−	−	−	−
6	48	+	−	−	−	−	−	−	−	−
7	48	+	−	−	−	−	−	−	−	−
8	48	+	+	+	−	−	−	−	−	−
9	48	+	+	−	−	−	−	−	−	−
10	48	+	+	+	+	+	+	+	+	+
11	72	+	+	−	−	−	−	−	−	−
12	72	+	−	−	−	−	−	−	−	−
13	72	+	−	−	−	−	−	−	−	−
14	72	+	+	+	−	−	−	−	−	+
15	72	+	+	+	+	+	+	+	+	+
16	96	+	−	−	−	−	−	−	−	−
17	96	+	+	+	+	+	+	+	+	+
18	96	+	+	+	+	+	+	+	+	+
19	96	+	−	−	−	−	−	−	−	−
20	96	+	+	+	−	+	+	−	−	+
21	120	+	−	−	−	−	−	−	−	−
22	120	+	+	+	+	+	+	+	−	−
23	120	−	−	−	−	−	−	+	+	−
24	120	+	+	+	−	−	+	−	+	+
25	120	+	+	+	+	+	+	+	+	+
26	144	+	+	+	+	+	+	+	+	+
27	144	+	+	+	+	+	+	+	+	+
28	144	+	+	+	+	+	+	+	+	+
29	144	+	+	+	+	+	+	+	+	+
30	144	+	+	+	+	+	+	+	+	+

[a] Plus and minus signs indicate positive and negative results, respectively, obtained from planting various 3-inch sections of the inoculated stem. Where a cutting showed infection it was considered to indicate that the virus had reached that section of the stem in its downward movement.

[b] Counting downward from inoculated top.

SUGAR BEET

Seedling Beets

Experiments with seedling beets were planned with a view to studying the rate of movement of virus in cotyledons under different conditions of temperature. Seedling plants were used that had the beginning of first true leaves and cotyledons more than 1 inch long. Leaf hoppers were caged singly on the tip of one cotyledon of each plant. The time at which each leaf hopper started to feed was noted and the feeding period was terminated after the desired interval by removing the leaf hopper. The plants were grown in 6-inch pots, 4 plants per pot. The pots were numbered consecutively. In all even-numbered pots the cotyledons on which the leaf hoppers fed were severed 1 inch from the point of feeding after the desired period allowed for virus movement. The odd-numbered pots were kept as controls and received the same treatment as the even-numbered pots, except that the cotyledons on which the leaf hoppers fed were not removed. The feeding periods of the leaf hoppers were 2, 3, and 5 minutes; the periods allowed for virus movement were 2, 3, 5, 10, and 15 minutes; and the air temperatures at which the various tests were made were roughly 60°, 85°, 110°, and 135° F. The results of these experiments are shown in table 8.

TABLE 8.—*Rate of movement of virus in cotyledons of young sugar-beet plants*

Temperature (°F.)	Leaf-hopper feeding period	Period allowed for virus to move 1 inch	Cotyledon removed			Cotyledon not removed		
			Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	Minutes	*Minutes*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
60	2	2	80	0	.0	79	0	.0
	3	3	80	0	.0	80	0	.0
	5	5	80	1	1.2	80	9	11.2
	5	10	66	5	7.5	71	11	15.4
	5	15	77	5	6.4	82	6	7.3
85	2	2	176	0	.0	183	9	4.9
	3	3	81	15	18.5	79	24	30.3
	5	5	70	8	11.4	76	19	25.0
	5	10	74	14	18.9	74	17	22.9
	5	15	73	13	17.8	75	21	28.0
	5	60	80	11	13.7	80	15	18.7
110	2	2	80	2	2.5	80	8	10.0
	3	3	78	6	7.7	80	11	13.7
	5	5	82	32	39.0	78	31	39.7
	5	10	68	18	26.4	64	12	18.7
	5	15	80	26	32.5	80	25	31.2
135	2	2	80	1	1.2	80	6	7.5
	5	5	40	8	20.0	40	10	25.0

Infection did not occur in any plant after the 2- and 3-minute feeding periods in the 60° series nor in any plant after the 2-minute feeding in the 85° series. Infection occurred in test plants and controls after all the other feeding periods and exposures in all series. As calculated from these data, the rates of movement were as follows: At 60°, 12 inches per hour; at 85°, 20 inches per hour; at 110°, 30 inches per hour; and at 135°, 30 inches per hour. However, considerable caution should be exercised in drawing conclusions from these calculations regarding the effect of temperature on the rate of virus movement. Although the calculated rate was lowest at 60°, the percentage of infection was also low. The low percentage of infection indicates a lower inoculative efficiency on the part of the vector, which in turn may mean that the minimum time required for infection to occur may be longer than at higher temperatures, thus correspondingly reducing the available period for virus movement. However, the results at 110° seem to furnish some support for the assumption that the virus moves more rapidly at this temperature than at 60° or at 85°, since in the 5-, 10-, and 15-minute exposures, as measured by the controls, the removal of the cotyledons on which the leaf hoppers fed seemed to have little influence on the percentage of plants which later developed disease; whereas at 60° and 85° the percentage of infection was considerably reduced by removing the cotyledons after exposure.

Beet Plants Having Several True Leaves

In seedling plants the determination of very rapid rates of virus movement is limited by the minimum duration of the required infection period and by the length of the cotyledons. In order to obtain a relation between time of infection and extent of movement which would permit the detection of rates of movement more rapid than 30 inches per hour, plants having leaves 3 to 10 inches long were used in a second experiment. Ten leaf hoppers were allowed to feed 6 minutes at the distal end of a leaf on each plant at a temperature of 85° to 100° F. In the odd-numbered pots the inoculated leaves were severed 1, 2, 3, etc., up to 10 inches from the point of inoculation 6

minutes after feeding started. The even-numbered pots were retained as controls. The experiment was run in three series (table 9). The results within a series are considered comparable, but one series is not strictly comparable with any other, because of differences in date of inoculation and in age of plants used.

The results of this experiment show a maximum downward movement of 6 inches in 6 minutes, or a rate of 60 inches per hour. An analysis of results indicates that even this rapid rate of movement does not represent the maximum rate attainable under the most favorable conditions for movement. Series 1, 2, and 3 of table 9 show considerably less infection in plants from which the inoculated leaf was removed than in the controls. This, however, did not hold in some of the subsequent tests made on very rapidly growing plants.

Experiments 1 and 2 of table 9 show the results of two additional tests on plants growing at different rates at the time of inoculation. The plants used in experiment 1 of this table were thrifty but were not growing at an excessive rate; those used in experiment 2 were in rich soil and were growing very rapidly. In experiment 1, infection in the test plants was considerably less than in the controls. Moreover, infection decreased as the distance of required virus movement increased. The results of this experiment, if standing alone, would indicate (1) that there was a rather uniform decrease in infection as the length of leaf removed was increased and (2) that the virus would not move more than 4 inches in 6 minutes.

TABLE 9.—*Virus movement in leaves of young sugar-beet plants*

Series or experiment	Length of leaf removed	Inoculated leaf not removed			Inoculated leaf removed			Calculated rate of virus movement per hour
		Plants inoculated	Plants infected		Plants inoculated	Plants infected		
	Inches	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Inches*
Series 1	1	120	56	46.6	120	27	22.5	10
	2	120	64	53.3	120	33	27.5	20
	3	120	50	41.6	120	20	16.6	30
Series 2	4	140	38	27.1	140	15	10.7	40
	5	140	46	32.8	140	15	10.7	50
	6	140	47	33.5	140	17	12.1	60
Series 3	7	40	10	25.0	40	0	0	----------
	8	40	11	27.5	40	0	0	----------
	9	40	10	25.0	40	0	0	----------
	10	40	10	25.0	40	0	0	----------
Experiment 1	1	20	17	85	20	8	40	10
	2	20	13	65	20	6	30	20
	3	20	15	75	20	3	15	30
	4	20	11	55	20	1	5	40
	5	20	7	35	20	0	0	----------
	6	20	6	30	20	0	0	----------
Experiment 2	1	20	13	65	20	8	40	10
	2	20	14	70	20	10	50	20
	3	20	10	50	20	10	50	30
	4	20	13	65	20	11	55	40
	5	20	12	60	20	11	55	50
	6	20	12	60	20	9	45	60

In rapidly growing plants, results were quite different. The removal of the inoculated leaf after 6 minutes did not appreciably decrease the percentage of infection as measured by the controls. This was true regardless of whether 1, 2, 3, 4, 5, or 6 inches of leaf was removed, and the chances, therefore, that the virus would move 6 inches in 6 minutes were about the same as that it would move 1 inch in 6 minutes. It seems probable that under the conditions of this experiment virus could have been shown to move more than 6 inches in 6 minutes had longer leaves been available.

DISCUSSION

The results obtained in the various experiments outlined in this paper are interpreted as indicating a very intimate relationship between the curly-top virus and the phloem tissue of affected plants. Virus is introduced into the phloem by its insect vector. It moves downward from the point of inoculation toward the root system at a very rapid rate. Results of leaf-hopper feeding experiments indicate that the virus concentration in exudate, believed to be derived largely from the phloem, is high as compared with that in expressed juice from the entire beet. Virus apparently does not pass from the xylem into the phloem or any other tissue in sufficient quantities to cause systemic infection. After it is established in the phloem the evidence indicates that it passes into adjacent parenchyma tissue only in very limited amounts in the beet, and experiments have failed to demonstrate that it ever occurs in the parenchyma of two species of tobacco. The evidence supporting these conclusions may be worthy of further study.

It is considered probable that the close association of the virus with the phloem tissue may have a bearing on the difficulty encountered in obtaining infection by mechanical inoculation. The ordinary methods of mechanical inoculation undoubtedly can be depended upon to introduce virus into parenchymatous tissue of various kinds, since infection is induced readily with other viruses by these methods. Artificial-inoculation experiments indicate that introduction of virus into such parenchyma cells as the leaf hairs of beet, tobacco, squash, or bean, never produces infection. Since the virus does not set up a systemic infection after its introduction into parenchymatous cells, only two other possible courses are open to it. It must either remain in these cells and fail to set up a systemic infection or it must be inactivated by substances resulting from cell injury or by normal constituents of parenchymatous cells.

In the light of the information now available, it seems probable that if artificial infection is to be obtained in any appreciable percentage of the plants inoculated the virus must be introduced directly into the phloem. Clearly the introduction of virus into phloem by mechanical means is attended with difficulty. Needles that are available for introducing virus into plants are large enough to crush many cells, and it is probable that the phloem is so badly injured in the process of inoculation that the virus does not often become established. Even if needles sufficiently fine to penetrate the phloem without causing excessive injury were available, they would probably still fall far short of the insect vector in effectiveness. Such a needle would necessarily carry the virus on the surface, where it would be exposed to the action of contents of parenchyma cells and to depletion in the passage of the needle through tissue exterior to the bundle. Once the needle penetrated the phloem, a portion of the virus might be liberated. If the needle were withdrawn, the phloem content, being under a positive pressure, would pass quickly into the cavity that was left, probably carrying a part or all of the virus deposited in the phloem back into the region of parenchyma.

The best available method of artificial inoculation seems crude when compared with the refinements introduced by the insect vector. The leaf hopper's stylets are extremely slender and seem to find the

phloem with remarkable accuracy. As the stylets pass through the parenchymatous regions exterior to the phloem, a protective sheath is laid down which may effectually exclude contents of surrounding cells. After the phloem is punctured the insect remains in a feeding position for appreciable periods and may leave considerable deposits of sheath material in the vascular area. As the stylets are removed the puncture is probably completely plugged by sheath material.

Since virus is introduced into the phloem directly by the leaf hopper, its movement to certain parts of the beet plant probably starts immediately and is very rapid. Probably the phloem of the growing point is invaded in a few hours and the entire phloem network of the plant is invaded in a few days. Evidence indicates that under some conditions the virus is closely restricted to the phloem, under other conditions it may escape into the intercellular spaces of the parenchyma. This is shown by the occurrence of drops of exudate having a high virus content on the petioles of badly diseased beets. Esau (*13*) has shown that this exudate moves from the vicinity of the phloem to the exterior of the petiole through the intercellular spaces. The causes of such movements are not clearly understood.

Crafts (*9*) states that the phloem is normally under a positive pressure and suggests that the cambium on one side and the starch sheath on the other limit lateral movement of phloem content. It is possible that phloem necrosis and the presence of regenerative tissue may interfere with the movement of solutes and increase the pressure in the phloem of diseased beets. This increased pressure may be sufficient to force phloem content through the limiting layers into the intercellular spaces of the parenchyma and to the surface of the petiole, or, as Esau has suggested, the presence of the virus may render the limiting layers more permeable to phloem content. If the cells of the limiting layer are rendered more permeable it seems probable that there might be a seepage of virus from phloem tissue throughout the plant. If the virus is not extruded through breaks in the limiting layer it may pass through cells that are rendered more permeable. Whether the virus is able to pass from the intercellular spaces into the cells of the parenchyma seems doubtful. The failure to obtain infection by introducing virus into parenchyma cells and the rapid inactivation of virus in expressed juice point to parenchyma as being a very poor medium for virus. The failure of leaf hoppers to acquire virus from any type of parenchyma except that immediately below the crown and in one instance from parenchyma of the petiole indicates that the virus content of parenchyma is very low. The relatively small amount of virus obtained by leaf hoppers from expressed juice as compared to that obtained from phloem exudate indicates that the juice is low in virus content or that the leaf hoppers are much less efficient in acquiring virus from expressed juice than from phloem exudate.

The weight of evidence seems to indicate that the virus content of parenchyma of beet is very low. The virus present in parenchyma may occur largely or exclusively in the intercellular spaces.

It seems probable that the virus may be even more closely restricted to the phloem in the two species of tobacco tested than in sugar beet. This is indicated by results of experiments in which the virus was held in inoculated parts in plants in which its further dispersal was dependent upon its ability to pass through a small amount of parenchym-

atous tissue. In most instances this parenchymatous bridge was composed of only a few cells, as in plants in which two rings, an internal and an external, 1 inch apart, were placed in an internode. In plants having the internal ring above the external the virus would move down the stem in the external phloem 1 inch past the internal ring, thus affording an area equal to the linear distance between the rings multiplied by the circumference of the internal ring for inward movement of virus through the wood or medullary rays to the internal phloem in which it could pass on down the stem. In plants having the positions of the two rings reversed the required movement would be from the internal to the external phloem through the wood or medullary rays. When it is considered that in these experiments active virus was held in the parts above the rings, in some instances for more than a year, with no leakage whatever of virus across the rings, the effectiveness of this barrier is apparent. To explain this phenomenon it is necessary again to assume that the virus does not pass out of the phloem or that it is very quickly inactivated by contact with normal cells of other tissues.

The close association of the curly-top virus with phloem tissue may have an important bearing on the failure of beet seeds to transmit disease. Artschwager (*1*) has shown that in the development of the beet seed there is no direct vascular connection between the mother plant and the young sporophyte. Therefore, materials that enter the embryo must do so by diffusing through a layer of meristematic or parenchymatous tissue. Results of experiments showing a lack of movement of virus through meristem and parenchyma indicate that such a layer would offer a formidable barrier to passage of the virus into the embryo, and might easily account for the absence of seed transmission of this disease.

Just how far it is safe to venture in applying this principle to other virus diseases is difficult to say. It would seem to go far toward explaining lack of seed transmission in all diseases in which the virus is restricted to the phloem. It may be important for other virus diseases. Nelson (*20*) has suggested such an explanation for the erratic transmission of bean mosaic. The virus of this disease is not known to be restricted to the phloem. It occurs in only a part of the seeds from diseased plants. In studying the development of the bean seed, Nelson found no direct vascular connection between the mother plant and the embryo. He suggests that the virus may be able to pass into the embryo through the nonvascular-containing layer only when this layer is in a meristematic stage of development. The failure of certain seeds to carry virus is accounted for by assuming an incomplete distribution of the virus in the phloem. Even with a complete virus invasion of the phloem, which may seem more likely in plants grown from diseased seeds, it would seem reasonable to suppose that the passage of virus through meristem or parenchyma of this type may be a hazardous one and successfully accomplished only under certain conditions. It is possible that the meristematic or parenchymatous layer of tissue separating the mother plant from the embryo may offer a structural or chemical barrier to virus passage even with such viruses as that of tobacco mosaic, which is known to occur in certain types of parenchymatous tissue.

The rate of movement of virus in sugar-beet leaves is so rapid as to call for a consideration of the mechanics of this phenomenon. Since it seems fairly obvious from data already presented that this movement

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occurs in the phloem, the possibilities inherent in the solution of this problem are all the more interesting. Under conditions of the greatest measured rate of virus movement in sugar beet (6 inches in 6 minutes) it seems safe to conclude that there is no appreciable multiplication of virus in the plant in so short a time, and it would not seem that multiplication would be an appreciable accelerating force in movement. That such movement may result from any autonomous effort on the part of the virus particles seems out of the question, since this rate is several times greater than the most rapid movement of the swiftest moving micro-organisms known. Simple diffusion is infinitely slower than the measured rate of virus movement. Diffusion accelerated by protoplasmic streaming in the sieve tubes could account for no rate approaching maximum movement.

Certainly none of the foregoing theories will account for this rate of movement, regardless of whether virus is considered to be a living organized entity or a chemical compound. In spite of these facts it is now pretty definitely known to physiologists that certain substances move in phloem at a very rapid rate of speed. Mason and Maskell (*19*) calculate a rate of movement of sugars in the cotton plant equal to the rate of diffusion of molecules of that size in air. Crafts (*10*), using figures derived from growth increase in pumpkin and cucumber over a stated period, estimates an average linear rate of movement of 0.292 and 0.235 cm per minute, respectively, of materials through the stem into the fruits. By cutting stems of cucumber and measuring phloem exudate, he found that a calculated rate of movement of phloem content varying between 3.64 and 8.62 cm per minute could be induced. Crafts (*9*), in discussing food translocation, suggests that a mass movement of elaborated food materials takes place in the phloem. This mass movement he conceives as being dependent on the creation of a pressure gradient due normally to the increased osmotic pressure in the more active photosynthetic areas of the plant. This high osmotic pressure accelerates the intake of water, which in turn increases the hydrostatic pressure in the phloem and causes phloem content to move to parts where the pressure is lower. In such a system each compound would not move independently of other compounds as in simple diffusion, but would move at a rate approximating that of the mass as a whole, assuming no selective interference in the path of movement.

Accepting this hypothesis of food translocation, we have available a hypothesis of virus movement which seems to satisfy all the known conditions of such movement. In a virus movement of 6 inches in 6 minutes it seems unlikely, as stated previously, that virus increase would be an important factor under any conditions. Therefore virus concentration during the first few minutes after introduction is probably very low, and the virus itself could not function to increase appreciably the osmotic concentration of materials at the point of introduction. However, if the hydrostatic pressure of the phloem at the point of virus introduction were already high because of an abundance of photosynthates, and these photosynthates were being transported at a very rapid rate, the introduced virus particles would be carried along at a rate corresponding to the rate of food flow, provided of course that mechanical interference were the same for each. Under such conditions the virus would move at the same rate and in the same direction as elaborated foods in the phloem and would in fact be an indicator of the rate and direction of food translocation.

SUMMARY AND CONCLUSIONS

Sugar-beet plants were induced by various treatments to take up juice of beets affected with curly top through the water-conducting channels, but since none of the plants so treated became infected, it is evident that the curly-top virus does not pass from the tracheae into cells or tissues where it can become established and initiate pathologic symptoms.

Several other methods of inoculation were tried with inocula prepared in different ways, but an appreciable percentage of infection was obtained only when the phloem exudate of a curly-top beet was used as the inoculum.

The vector of the curly-top virus, *Eutettix tenellus*, feeds on the leaf veins, and its mouth parts usually penetrate the phloem region. As the mouth parts are inserted the insect lays down a sheath of apparently gelatinous material which completely incases the stylets. This sheath may seal off all cells penetrated that are external to the phloem, and thereby protect the virus as it is passed into or drawn out of the phloem by the leaf hopper.

The higher mortality rate in groups of leaf hoppers on parenchyma tissue, as compared with mortality in groups on tissue containing vascular elements, indicates that parenchymatous tissue does not serve as a favorable source of food. However, leaf hoppers having access to parenchyma lived longer than those given neither food nor water, indicating that they extracted a certain amount of material from parenchyma. Leaf hoppers given access to parenchyma tissue of petioles and crowns and to pith and immature seeds, all from diseased beets, and then caged in groups of 5 on healthy beet seedlings, infected only 10 of 428 plants. An equal number of insects from tissue containing vascular elements infected 210 of 428 plants. These results supplement other evidence supporting the view that virus is concentrated in the phloem and is present only in relatively small amounts in the parenchyma. Since in sugar beets affected with curly top, phloem content escapes into the intercellular spaces of parenchyma tissue surrounding the vascular bundles, it is suspected that the virus recovered from the parenchyma may have been derived from escaped phloem content.

The exudate occurring on the petioles and blades of beets affected by curly top and that from the cut surface of affected beets has a high virus content. Evidence indicates that the exudate that occurs naturally on the petioles and blades is derived largely from the phloem and that the exudate from the cut surface of the beet is derived from the phloem except as it may be contaminated by an undetermined amount of material from the xylem and from injured cells mainly parenchymatous in nature.

Healthy sugar-beet and tobacco plants were infected by grafting diseased plants on them. Beets were not infected when union of the grafted plants did not result. In the grafted tobacco plants a definite union consisting of meristematic tissue was found after 3 days. In unions 7, 8, and 9 days old, tracheal elements were apparently mature and strands of elongated cells paralleling the tracheae probably included functional phloem. No infection resulted until the seventh day. Infection increased from 27 percent on the seventh day to 100 percent on the twelfth day. In view of the fact that the virus does not gain effective entrance through the tracheae and did not in these

experiments pass through meristematic or parenchymatous tissue, it must have passed the graft union through the phloem elements.

Ringing experiments with *Nicotiana tabacum* and *N. glauca* showed that the virus passes all rings bridged by an uninterrupted path of phloem, internal or external or internal and external combined. The virus failed to move past the rings when the internal and external phloem were removed at the same level. Interruption of phloem continuity by rings placed at different levels in an internode prevented the passage of virus in 20 of 27 *N. tabacum* plants and in 26 of 30 *N. glauca* plants. Serial sections of the ringed area in all the plants in which the virus passed the rings showed in each case one or more areas of regenerated tissue connecting the internal and external phloem through the woody cylinder. In view of these findings it seems probable that virus does not move longitudinally or radially through any of the normal elements of the woody cylinder and that dispersal in these two species of tobacco is dependent on the presence of continuous phloem elements.

The movement of the curly-top virus in tobacco is relatively slow as compared to the movement in sugar beet. The fastest movement observed in tobacco was downward from the point of inoculation at the top of the plant to a point 24 inches below in 48 hours; a rate of movement of one half inch per hour.

In sugar beet the virus moves at a much more rapid rate. At air temperatures of approximately 85°, 110°, and 135° F. the virus moved outward in cotyledons from the point of inoculation a distance of 1 inch in 2 minutes. In larger beets the virus moved downward from the point of inoculation at the distal end of a leaf to a point 6 inches below in 6 minutes, a rate of movement of 60 inches per hour. These rapid movements of virus evidently occur in the phloem and it is suggested that they indicate a rapid translocation of plant materials. For these reasons virus may prove useful as an indicator in studies on the movement of elaborated foods.

LITERATURE CITED

(1) Artschwager, E.
1927. development of flowers and seed in the sugar beet. Jour. Agr. Research 34: 1–25, illus.

(2) Bennett, C. W.
1927. virus diseases of raspberries. Mich. Agr. Expt. Sta. Tech. Bull. 80, 38 pp., illus.

(3) ———
1932. further observations and experiments with the mosaic diseases of raspberries, blackberries, and dewberries. Mich. Agr. Expt. Sta. Tech. Bull. 125, 32 pp., illus.

(4) Böning, K.
1928. beiträge zum studium der infektionsvorgänge pflanzlicher viruskrankheiten. Ztschr. Parasitenk. 1: 198–230, illus.

(5) Brandes, E. W.
1923. mechanics of inoculation with sugar-cane mosaic by insect vectors. Jour. Agr. Research 23: 279–284, illus.

(6) Büsgen, M.
1891. der honigtau. Ztschr. Naturw. (n.F. 18) 25: 339–428, illus.

(7) Caldwell, J.
1930. the physiology of virus diseases in plants. I, the movement of mosaic in the tomato plant. Ann. Appl. Biol. 17: 429–443, illus.

(8) Carsner, E., and Stahl, C. F.
1924. progress report on curly-top of the sugar beet. (Abstract) Phytopathology 14: 122–123.

(9) Crafts, A. S.
1931. movement of organic nutrients in plants. Plant Physiol. 6: 1–38, illus.
(10) ———
1932. phloem anatomy, exudation, and transport of organic nutrients in cucurbits. Plant Physiol. 7: 183–225, illus.
(11) Dana, B. F.
1932. some experiments with mechanical transmission of the curly-top virus. (Abstract) Phytopathology 22: 997–998.
(12) Davidson, J.
1923. biological studies of aphis rumicis linn. the penetration of plant tissues and the source of the food supply of aphids. Ann. Appl. Biol. 10: 35–54, illus.
(13) Esau, K.
1933. pathologic changes in the anatomy of leaves of the sugar beet, beta vulgaris l., affected by the curly-top disease. Phytopathology 23: 679–712, illus.
(14) Fife, J. M.
1932. a method of artificially feeding the sugar-beet leafhopper. Science (n.s.) 75: 465–466, illus.
(15) Holmes, F. O.
1930. local and systemic increase of tobacco mosaic virus. Amer. Jour. Bot. 17: 789–805, illus.
(16) Horsfall, J. L.
1923. the effects of feeding punctures of aphids on certain plant tissues. Pa. Agr. Expt. Sta. Bull. 182, 22 pp., illus.
(17) King, W. V., and Cook, W. S.
1932. feeding punctures of mirids and other plant-sucking insects and their effect on cotton. U.S. Dept. Agr. Tech. Bull. 296, 12, pp., illus.
(18) McCubbin, W. A., and Smith, F. F.
1927. rate of virus spread in tomato plants. Science (n.s.) 66: 486–487.
(19) Mason, T. G., and Maskell, E. J.
1928. studies on the transport of carbohydrates in the cotton plant. II. the factors determining the rate and the direction of movement of sugars. Ann. Bot. [London] 42: 571–636, illus.
(20) Nelson, R.
1932. investigations in the mosaic disease of bean (phaseolus vulgaris l.) Mich. Agr. Expt. Sta. Tech. Bull. 118, 71 pp., illus.
(21) Severin, H. H. P.
1921. minimum incubation periods of causative agent of curly leaf in beet leafhopper and sugar beet. Phytopathology 11: [424]–429, illus.
(22) ———
1924. curly leaf transmission experiments. Phytopathology 14: [80]–93, illus.
(23) Smith, F. F.
1933. the nature of the sheath material in the punctures produced by the potato-leaf hopper and the three-cornered alfalfa hopper. Jour. Agr. Research 47: 475–485.
(24) ——— and Poos, F. W.
1931. the feeding habits of some leaf hoppers of the genus empoasca. Jour. Agr. Research 43: 267–285, illus.
(25) Smith, K. M.
1926. a comparative study of the feeding methods of certain hemiptera and of the resulting effects upon the plant tissue, with special reference to the potato plant. Ann. Appl. Biol. 13: 109–139, illus.
(26) ———
1931. virus diseases of plants and their relationship with insect vectors. Biol. Rev. 6: 302–344.
(27) Storey, H. H.
1928. transmission studies of maize streak disease. Ann. Appl. Biol. 15: 1–25, illus.

CHEMICAL COMPOSITION AND YIELD OF THE ALASKA PEA AS INFLUENCED BY CERTAIN FERTILIZERS AND BY THE STAGE OF DEVELOPMENT[1]

By SAMUEL L. JODIDI, *physiologist*, and VICTOR R. BOSWELL, *senior horticulturist*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It is believed by many canners and growers of peas for the cannery that if potash in appreciable amounts is present in the fertilizer applied to a pea crop the peas will mature earlier and be harder, more starchy, and of lower quality at a given stage of development than if no potash or only a small amount is used. Boswell (*3*, *4*)[2] conducted fertilizer experiments in three different parts of Maryland over a period of 3 years and used, among other materials, muriate of potash alone and in combination with nitrogen and phosphorus. All treatments were duplicated each year. Although he made no chemical analyses of the peas, field observations led him to conclude (*4*, *p. 352*) that—

> Muriate of potash, alone or in combination with nitrogen and phosphorus, has given no consistent results with respect to yield, rate of maturity, or apparent condition of the crop at harvest. The opinion that potash hastens maturity and lowers the quality of the crop has not been borne out in this work.

Later Sayre and his associates (*23*) made exhaustive chemical and physical examinations of peas grown under a great variety of fertilizer treatments in the field and in water cultures. They devoted special attention to the effect of potash upon rate of maturity and quality of peas. In their conclusions it is stated: "In spite of this wide variety of conditions, only minor differences in quality of peas *of the same stage of growth* have been obtained." They further conclude: "Thus far, the belief that potash has a harmful effect upon the quality of the crop appears to be ill-founded."

Earlier work of one of the present writers (*4*) indicated the desirability of determining by more accurate methods than had previously been used the effect of potash and other fertilizer constituents upon the development of the pea as indicated by its chemical composition. Nitrogen fertilizers appeared to be of special interest, since earlier observations had suggested that a generous use of available nitrogen tends to delay maturity and to improve the quality of the pea. Accordingly, studies were made to determine the effect of certain fertilizers and of the stage of development on the composition and yield of peas.

GROWING AND HANDLING EXPERIMENTAL MATERIAL

FIELD-PLOT METHODS

The Alaska variety of pea (*Pisum sativum* L.) was used in these studies. Work was carried on at the Arlington Experiment Farm,

[1] Received for publication Dec. 27, 1933; issued June 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 735.

Rosslyn, Va., in 1930 and 1931. All treatments were in duplicate, and each consisted of an application of a single fertilizer constituent. The treatments and rates of application per acre were as follows:

N.—Nitrate of soda 400 pounds and sulphate of ammonia 280 pounds (about 116 pounds of nitrogen).
P.—Superphosphate 1,000 pounds (about 160 pounds of phosphoric acid).
K.—Muriate of potash 300 pounds (about 144 pounds of potash).
C.—Check; no treatment.

The quantities of fertilizers used per acre were in excess of those employed in growing peas for the cannery, the object being to produce measurable differences in the composition of the products. In order to overcome as far as possible any definite gradient in soil conditions that might exist, the eight plots were arranged as follows:

Potash	Check
Phosphorus	Nitrogen
Check	Phosphorus
Nitrogen	Potash

The fertilizing materials were applied by hand after the soil had been plowed and disked once. They were then worked in by further disking and harrowing, care being taken to avoid dragging soil from one plot to another. The peas were sown with a garden drill at a depth of 1 to 1½ inches.

In 1930 the plots were located on a moderately fertile area of artificial land which had been dredged from the Potomac River and which was apparently of a silt-loam character. Each plot was 53 by 33 feet. The rows were 14 inches apart, sown at the rate of approximately 4 bushels of seed per acre. A border of 1 row along the sides and a width of 1 foot at the ends of each plot was left unharvested. In 1931 the same plots and treatments were used as in 1930. One tier of 4 plots was sown in rows 7 inches apart, at the rate of 4 bushels of seed per acre; the other tier was sown in 14-inch rows, at the rate of 2 bushels per acre. In 1930 the fertilizers were applied and seed sown on March 18, and the peas were harvested on May 28 and on June 2. In 1931 the plots were fertilized and sown on April 10 and the peas harvested on June 6 and June 10.

HARVESTING, GRADING, AND DRYING

In both 1930 and 1931 a power-driven pea huller, a hand-driven grader, and a specially constructed drier not only permitted the use of large and adequate samples but also very greatly reduced the time from harvest to complete dryness of the material.

The drier consisted of an asbestos-board cabinet 6 feet high, 4½ feet wide, and 2½ feet deep, inside measurement, with the entire front made of double doors which opened at the center. The outside was covered with a one half inch layer of builders' insulating material. Ten shallow trays 4 feet long and 2½ feet wide rested upon horizontal iron-rod supports. The bottoms of the trays were of 8-mesh hardware cloth. Each tray was supported by three three-eighth inch rods

which prevented the sagging of the center of the large screen-wire bottom. There was a 3-inch "head space" between trays. The opposite ends of alternate trays were placed against the side walls when the drier was in operation, thus forcing the hot air to travel over each tray that carried material to be dried.

Immediately to one side of the cabinet and connected with it by an opening near the base were two low-form, five-column, common steam radiators each of 50 square feet radiation. Over these radiators, which were enclosed in an asbestos-board housing, a strong current of air was forced by a centrifugal blower of a capacity of 1,500 cubic feet per minute driven by a three-horsepower electric motor. With a steam pressure of 15 pounds per square inch in the radiators, a temperature of 60° to 65° C. could be maintained in the cabinet during the drying of a considerable mass of plant material. In the brief period of 3 to 4 hours 25 to 30 pounds of peas were dried to such a degree that they rattled when handled. The material to be dried had to be spread very thinly and uniformly on the trays and moved carefully about from time to time to obtain satisfactory results.

At each harvest date the peas from one half of each plot were gathered; all pods were removed from all plants scheduled for harvest on that date. The duplicate or second series of plots was harvested in reverse order to the first, since all plots could not be harvested simultaneously. In most instances the samples of shelled peas were placed in the drier within 3 to 4 hours after the pods were harvested.

FIELD RESULTS

YIELD

The yield data are of secondary importance, since the treatments were made for a specific purpose other than for increasing yields and were of a character that ordinarily would not be recommended in field practice. However, the yield data, presented in table 1, indicate the growth relationships due to the various treatments and the general level of nutritional conditions under which the studies were made. Individual plots of the duplicate treatments are designated A and B.

Even though small differences appear between the total yields of fresh shelled peas from the various treatments in 1930 and 1931, they are of no significance, for the differences between duplicates are in general greater than between treatments. The absence of material differences in yield is not what one might expect but possibly may be accounted for in part by (1) the high fertility of the soil to which treatments were applied, and (2) by injury from excessive amounts of readily soluble nutrient salts. The latter suggestion carries little weight because no definitely injurious effects were noted except in the nitrogen-treated plots of 1930. Furthermore, in market-garden areas peas are heavily fertilized without injury. A late frost in 1930 injured the nitrogen-treated plots rather severely, but the others were not appreciably harmed. Further evidence of the lack of fertilizer injury was afforded by yields of similar plots in 1931. Germination of the pea is known to be adversely affected by excessive concentrations of fertilizer salts, but no damage was noted in any of these plots.

TABLE 1.—*Yield per acre of shelled peas (fresh weight) as affected by potash, phosphoric acid, and nitrogen in 1930 and 1931*

Harvest and grade no.	Plot	Yield from treatment indicated			
		Check	Potash	Phosphoric acid	Nitrogen
1930 CROP					
Harvest of May 28:		*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	A	98	158	167	51
	B	118	112	179	135
2	A	182	146	218	79
	B	236	292	250	109
3	A	149	58	195	103
	B	136	374	213	73
4	A	39		48	19
	B	30	68	41	31
5	A			12	24
	B				
All grades	A	468	362	640	276
	B	520	846	683	348
Average, A and B		494	604	662	312
Harvest of June 2:					
1	A	85	75	86	71
	B	75	99	74	37
2	A	185	168	144	80
	B	140	212	141	120
3	A	313	234	601	391
	B	434	352	573	155
4	A	401	154	321	196
	B	185	328	567	173
5	A	145	80	68	
	B	112	136		
All grades	A	1,129	711	1,220	738
	B	946	1,127	1,355	485
Average, A and B		1,038	919	1,288	612
1931 CROP					
Harvest of June 6:					
1	A	104	164	215	122
	B	128	176	202	104
2	A	261	287	352	289
	B	303	512	444	303
3	A	234	157	192	265
	B	257	306	289	208
4	A	63	22	35	99
	B	40	84	42	36
All grades	A	662	630	794	775
	B	728	1,168	977	651
Average, A and B		695	899	886	713
Harvest of June 10:					
1	A	127	137	198	105
	B	320	212	351	260
2	A	402	382	414	316
	B	761	646	928	626
3	A	678	558	934	592
	B	943	1,166	994	882
4	A	542	462	724	580
	B	383	700	352	358
5	A	17	19	57	28
	B		22	8	12
All grades	A	1,766	1,558	2,327	1,621
	B	2,407	2,746	2,633	2,138
Average, A and B		2,087	2,152	2,480	1,880

In 1930 phosphoric acid produced a definite increase in yield. In 1931 the same plot with the same phosphorus treatment also showed an increase of appreciable magnitude (table 1).

It should be borne in mind that these increases in yield were obtained on a soil dredged from the river. In past years it had received moderate applications of manure and green manure and may have been relatively low in phosphorus. Other field experiments in nearby Maryland have not shown phosphorus to be markedly effective in increasing the yield of peas. As previously explained, the low yield of the nitrogen plot in 1930 is believed to have been due to frost injury while the peas were in an early stage of growth. In 1931 nitrogen and potash applications resulted in no significant differences in yield.

MATURITY

In this investigation the possible effect of nutrients upon rate of development and properties of the peas is of more interest than the effect upon yield.

In this work the rate of development or stage of maturity has been judged on the basis of distribution of sizes of peas in the pods. This is the basis used by canners' field men in determining when a field has attained the proper stage for harvesting. In order to reduce the expression of maturity to a single numerical value, the percentage of the yield of a plot constituted by each grade was multiplied by an arbitrary weighting, for convenience, equal to the standard grade number. The sum of these products of a single harvest is designated as a "maturity index" (*4*). Sayre and his associates (*23*) have suggested, since the present work was begun, that as an index to quality this maturity index is less reliable than their "quality index." The latter is calculated by summing the products obtained in multiplying the percentage of yield constituting each grade by the numerical value of the crushing test for that grade. However, since stage of development is commonly judged by distribution of sizes, and since this work was designed to study the pea as generally handled in field practice, the above-described maturity index is believed to be better adapted to the present investigation.

Table 2 shows the distribution of sizes of shelled peas of each harvest from each plot as well as the maturity indices for each plot and for the total yield of duplicate plots of each treatment. No appreciable consistent differences in maturity resulted from the treatments. The same was true when adjacent different treatments were compared in the field. In one comparison the difference was in favor of one plot, and, more often than not, the relationship was reversed in the second comparison. When the indices for the total plot area of each treatment are compared, they are found to be quite similar for any one harvest date.

There was no evident difference in the time of blossoming of the plants in any of the several plots. Thus, earlier observations in the field, and the reports of other investigators (*4*, *23*), appear to be confirmed under additional and quite different conditions as reported in this paper.

TABLE 2.—*Influence of potash, phosphoric acid, and nitrogen upon stage of maturity of peas at harvest in 1930 and 1931*

1930 CROP

Harvest and grade no.	Plot	Yield from treatment indicated			
		Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:		*Percent*	*Percent*	*Percent*	*Percent*
1 and smaller	A	30.98	43.70	26.61	18.37
	B	22.70	13.20	26.20	38.80
2	A	38.88	40.35	34.11	28.55
	B	45.29	34.48	36.60	31.40
3	A	31.79	15.93	30.50	37.52
	B	26.20	44.24	31.20	21.06
4	A	8.36	----	7.48	6.98
	B	5.80	8.07	5.97	8.74
5	A	----	----	1.89	8.58
	B	----	----	----	----
Maturity index	A	228	172	216	259
	B	215	247	217	200
Maturity index of total A and B		221	225	221	226
Harvest of June 2:					
1 and smaller	A	7.50	10.54	7.04	9.63
	B	7.99	8.78	5.44	7.61
2	A	16.42	23.63	11.78	10.81
	B	14.80	18.81	10.43	24.75
3	A	27.72	32.92	49.35	53.00
	B	45.85	31.20	42.27	31.90
4	A	35.53	21.69	26.30	26.56
	B	19.57	29.08	41.85	35.75
5	A	12.81	11.23	5.55	----
	B	11.83	12.09	----	----
Maturity index	A	330	300	312	293
	B	313	317	321	296
Maturity index of total A and B		322	310	316	296

1931 CROP

Harvest of June 6:					
1	A	15.71	26.02	27.08	15.74
	B	17.59	15.08	20.67	15.97
2	A	39.42	45.56	44.33	37.30
	B	41.60	43.82	45.44	46.55
3	A	35.36	24.92	24.19	34.20
	B	35.30	33.90	29.51	31.94
4	A	9.52	3.49	4.41	12.76
	B	5.49	7.19	4.30	5.53
Maturity index	A	239	206	206	244
	B	229	233	217	227
Maturity index of total A and B		233	224	212	236
Harvest of June 10:					
1	A	7.19	8.79	8.52	6.48
	B	13.30	7.72	13.33	12.15
2	A	22.75	24.51	17.82	19.49
	B	31.61	23.52	35.23	29.28
3	A	38.40	35.82	40.05	36.51
	B	39.20	42.48	37.72	41.26
4	A	30.69	29.67	31.15	35.78
	B	15.90	25.49	13.37	16.74
5	A	.96	1.20	2.45	1.73
	B	.00	.80	.34	.59
Maturity index	A	295	290	301	307
	B	258	288	252	264
Maturity index of total A and B		274	289	275	283

PHYSIOLOGICAL STUDIES

Chemical analyses in connection with studies similar to those herein recorded have often been reported and discussed on the basis of oven-dry matter. Such a method of presentation has its proper applications, but alone would not be suitable here for the following reasons: (1) The study was designed to show the development and composition of fresh peas in response to certain field practices, therefore the results must be considered as they apply to fresh material; (2) peas grown in the garden or for the cannery are consumed on the fresh-weight basis, i.e., in the moisture-containing condition, so their composition as affecting nutritional value should be expressed on that basis; and (3) the results of this work when expressed on the dry-weight basis lead to conclusions for the most part opposite to, or quite different from, those reached when analyses are expressed on the fresh-weight basis. Consequently, analytical data are presented on both a fresh- and a dry-weight basis, but are discussed mainly on the fresh-weight basis. Certain facts are revealed which heretofore have not been plainly evident from the work of others who have reported their results on the basis of oven-dry matter only.

DRY MATTER

After the peas had been harvested, weighed, shelled, and graded, they were subjected to preliminary drying at 60° to 65° C. for 24 hours in the specially designed forced-draft drying apparatus already described. After drying, the samples were left for a few days in a dry room with access to the air, before they were weighed. This was done to reduce errors that might arise from the absorption of moisture during subsequent handling and grinding. The samples were ground to pass a 40-mesh sieve, and stored in tightly stoppered bottles until analyzed. The moisture content of the air-dry samples that had been dried in a preliminary way in the forced-draft apparatus was determined by drying them in an electric oven at a temperature of 103° to 105°, until constant weight was obtained. The results are recorded in table 3.

Table 3 shows that the percentage differences in the dry-matter content of pea samples grown in differently treated plots are insignificant, being mostly smaller than the percentage differences between samples from duplicate plots. For instance, the mean dry-matter percentages of grade 2, 3, and 4 peas from the untreated plot shown by samples 1, 3, and 5 and their duplicate samples 2, 4, and 6 are 22.37 and 22.87, respectively, making a difference of 0.50 percent. On the other hand, the difference between the dry-matter content of samples 2, 4, and 6 from the untreated plot (22.87) and from the potash-treated plot (23.14) is only 0.27 percent.

TABLE 3.—*Percentage of oven-dry matter in the Alaska pea as affected by different fertilizers; determined on the basis of fresh weight, 1930 and 1931*

1930 CROP

Harvest and sample no.	Grade no.	Plot	Dry matter from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	20.46	21.06	20.73	20.68
2	2	B	21.03	21.05	21.34	21.03
		Average	20.74	21.05	21.03	20.85
3	3	A	22.73	23.56	22.30	21.74
4	3	B	23.48	23.21	23.34	22.84
		Average	23.10	23.38	22.82	22.29
5	4	A	23.92		23.97	22.78
6	4	B	24.10	25.16	25.19	23.63
		Average	24.01	25.16	24.58	23.20
		Average (1–6)	22.62	22.81	22.81	22.11
Harvest of June 2:						
7	2	A	23.78	24.48	25.17	22.81
8	2	B	24.90	24.44	25.69	24.84
		Average	24.34	24.46	25.43	23.82
9	3	A	27.70	27.76	27.88	25.25
10	3	B	28.45	28.03	29.08	28.47
		Average	28.07	27.89	28.48	26.86
11	4	A	29.41	29.53	30.27	29.33
12	4	B	31.17	29.92	31.39	29.76
		Average	30.29	29.72	30.83	29.54
		Average (7–12)	27.57	27.36	28.25	26.74
		Average (1–12)	25.09	25.30	25.53	24.43

1931 CROP

Harvest of June 10:						
13	2	A	20.15	20.08		20.94
14	2	B	21.01	21.20		20.96
		Average	20.58	20.64		20.95
15	3	A	24.89	24.81		25.57
16	3	B	25.30	26.05		25.21
		Average	25.10	25.43		25.39
17	4	A	28.57	28.11		28.83
18	4	B	27.64	28.66		29.37
		Average	28.11	28.39		29.10
		Average (13–18)	24.59	24.82		25.15

ASH CONTENT

The ashing of the pea samples was effected in an electric muffle at a dull red heat, until constant weight was obtained. The ash was white and fluffy. In no case was trickling allowed to occur. The percentages of ash in the various pea samples are recorded in table 4. In view of the importance of the ash content in foodstuffs (*5*), the following regularities are pointed out.

The ash content was greater in the riper large-sized peas than in the unripe small-sized peas from both the 1930 and 1931 crops, although it did not increase in proportion to the dry matter. This

was true of all samples from a single plot at one harvest with but one exception. Another regular trend revealed by the ash results is that without exception peas of the same grade from the same plot but harvested later had a larger ash content than those harvested earlier. This increase in ash in the later harvested peas is most closely associated with increase in total dry matter; however, on the dry-weight basis the ash content ordinarily decreases with the size and age of the peas. It seems reasonable to ascribe this decrease to the fact that as the growth of the peas progresses the proportions of organic reserve substances such as carbohydrates and proteins increase more rapidly than the proportions of ash from the soil.

TABLE 4.—*Percentage of total ash in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Harvest and sample no.	Grade no.	Plot	Ash from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.771	0.811	0.771	0.781
2	2	B	.751	.816	.785	.776
		Average	.761	.813	.778	.778
3	3	A	.816	.841	.800	.787
4	3	B	.794	.850	.822	.827
		Average	.805	.845	.811	.807
5	4	A	.854	----------	.851	.793
6	4	B	.816	.900	.881	.827
		Average	.835	.900	.866	.810
		Average (1–6)	.800	.844	.818	.798
Harvest of June 2:						
7	2	A	.855	.862	.825	.840
8	2	B	.859	.932	.860	.832
		Average	.857	.897	.842	.836
9	3	A	.942	.922	.911	.883
10	3	B	.934	1.026	.922	.922
		Average	.938	.974	.916	.902
11	4	A	.982	.978	.962	.997
12	4	B	.992	1.069	.986	.955
		Average	.987	1.023	.974	.976
		Average (7–12)	.927	.965	.911	.905
		Average (1–12)	.863	.910	.864	.851

1931 CROP, FRESH WEIGHT

Harvest of June 10:						
13	2	A	0.915	0.915	----------	0.923
14	2	B	.907	.906	----------	.959
		Average	.911	.911	----------	.941
15	3	A	1.015	.987	----------	1.026
16	3	B	.986	.978	----------	1.024
		Average	1.001	.983	----------	1.025
17	4	A	1.110	1.074	----------	1.167
18	4	B	1.039	1.047	----------	1.080
		Average	1.075	1.061	----------	1.124
		Average (13–18)	.995	.985	----------	1.030

TABLE 4.—*Percentage of total ash in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Contd.

1930 CROP, OVENDRY WEIGHT

Harvest and sample no.	Grade no.	Plot no.	Ash from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	3.77	3.85	3.72	3.78
2	2	B	3.57	3.88	3.68	3.69
		Average	3.67	3.87	3.70	3.74
3	3	A	3.59	3.57	3.59	3.62
4	3	B	3.38	3.66	3.52	3.62
		Average	3.49	3.62	3.56	3.62
5	4	A	3.57		3.55	3.48
6	4	B	3.37	3.58	3.50	3.50
		Average	3.47	3.58	3.53	3.49
		Average (1–6)	3.54	3.71	3.59	3.62
Harvest of June 2:						
7	2	A	3.60	3.52	3.28	3.68
8	2	B	3.45	3.81	3.35	3.35
		Average	3.53	3.67	3.32	3.52
9	3	A	3.40	3.32	3.27	3.50
10	3	B	3.28	3.66	3.17	3.24
		Average	3.34	3.49	3.22	3.37
11	4	A	3.34	3.31	3.18	3.40
12	4	B	3.18	3.57	3.14	3.21
		Average	3.26	3.44	3.16	3.31
		Average (7–12)	3.38	3.53	3.23	3.40
		Average (1–12)	3.46	3.61	3.41	3.51

1931 CROP, OVEN-DRY WEIGHT

Harvest of June 10:						
13	2	A	4.27	4.21		4.13
14	2	B	4.06	4.02		4.32
		Average	4.17	4.12		4.23
15	3	A	3.81	3.75		3.80
16	3	B	3.69	3.57		3.88
		Average	3.75	3.66		3.84
17	4	A	3.69	3.61		8.82
18	4	B	3.54	3.46		3.53
		Average	3.62	3.54		3.68
		Average (13–18)	3.84	3.77		3.91

The difference between the ash content of peas grown in fertilized and unfertilized soil is rather insignificant, in most instances being about equal to, or even less than, the difference in the ash content of peas grown in duplicate plots. The differences in mean ash content of the phosphorus- or nitrogen-treated lots as compared with the untreated ones are certainly insignificant. It appears, however, that the potash-treated plots harvested May 28, 1930, showed a significant but small increase over the checks. In the harvests of June 2, 1930, and June 10, 1931, when the peas were at the stage usually harvested for the cannery, this difference was less striking and, indeed, was of

doubtful consequence. The mean ash content of the samples from the phosphorus- and nitrogen-treated plots of this latter harvest are in remarkably close agreement with the check.

For the qualitative examination of the ash a few grams of the peas, grade 3, which were grown in soil not treated with fertilizer (table 4, no. 3, fresh-weight basis) were ashed in an electric muffle oven. The ash obtained was partly insoluble in cold and hot water but dissolved readily with the addition of a few drops of hydrochloric, nitric, sulphuric, or phosphoric acid. The ash was found to contain the following elements: Aluminum (large amount); iron, both ferric and ferrous (very little); calcium, magnesium, potassium (large amount); sodium (trace); the acids sulphuric, phosphoric (large amount), and hydrochloric (trace).

ETHER EXTRACT

Ordinarily 5- or 10-g portions of the finely ground peas were dried in an oven at 100° C. for 1 hour, after which they were transferred to fat-free paper thimbles and covered with fat-free cotton. The thimbles were than placed in the Soxhlet extraction apparatus, in which the substance was extracted with anhydrous ether for 5 hours; the ether was then driven off, the residue in the previously weighed extraction flask dried at 100° for 1 to 2 hours, cooled, and weighed.

TABLE 5.—*Percentage of ether-soluble substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Ether-soluble substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.344	0.411	0.346	0.372
2	2	B	.318	.440	.414	.385
		Average	.331	.425	.380	.378
3	3	A	.382	.396	.370	.348
4	3	B	.385	.427	.387	.375
		Average	.383	.411	.378	.361
5	4	A	.335	----------	.347	.369
6	4	B	.349	.433	.347	.359
		Average	.342	.433	.347	.364
		Average (1–6)	.352	.421	.368	.368
Harvest of June 2:						
7	2	A	.378	.392	.373	.383
8	2	B	.428	.411	.383	.405
		Average	.403	.401	.378	.394
9	3	A	.421	.419	.398	.381
10	3	B	.435	.460	.419	.407
		Average	.428	.439	.408	.394
11	4	A	.424	.446	.412	.423
12	4	B	.436	.464	.424	.432
		Average	.430	.455	.418	.427
		Average (7–12)	.420	.432	.401	.405
		Average (1–12)	.386	.427	.384	.386

TABLE 5.—*Percentage of ether-soluble substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight*—Con.

Sample no.	Grade no.	Plot	Ether-soluble substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
1931 CROP, FRESH WEIGHT						
Harvest of June 10:			*Percent*	*Percent*	*Percent*	*Percent*
13	2	A	0.388	0.361		0.346
14	2	B	.395	.368		.333
		Average	.392	.365		.340
15	3	A	.469	.410		.405
16	3	B	.433	.392		.375
		Average	.451	.401		.390
17	4	A	.493	.464		.458
18	4	B	.476	.472		.459
		Average	.485	.468		.459
		Average (13–18)	.442	.411		.396
1930 CROP, OVEN-DRY WEIGHT						
Harvest of May 28:						
1	2	A	1.68	1.95	1.67	1.80
2	2	B	1.51	2.09	1.94	1.83
		Average	1.60	2.02	1.81	1.82
3	3	A	1.68	1.68	1.66	1.60
4	3	B	1.64	1.84	1.66	1.64
		Average	1.66	1.76	1.66	1.62
5	4	A	1.40		1.45	1.62
6	4	B	1.45	1.72	1.38	1.52
		Average	1.43	1.72	1.42	1.57
		Average (1–6)	1.56	1.86	1.63	1.67
Harvest of June 2:						
7	2	A	1.59	1.60	1.48	1.68
8	2	B	1.72	1.68	1.49	1.63
		Average	1.66	1.64	1.49	1.66
9	3	A	1.52	1.49	1.43	1.51
10	3	B	1.53	1.64	1.44	1.43
		Average	1.53	1.57	1.44	1.47
11	4	A	1.44	1.51	1.36	1.44
12	4	B	1.40	1.55	1.35	1.45
		Average	1.42	1.53	1.36	1.45
		Average (7–12)	1.53	1.58	1.43	1.52
		Average (1–12)	1.55	1.70	1.53	1.60
1931 CROP, OVEN-DRY WEIGHT						
Harvest of June 10:						
13	2	A	1.81	1.66		1.55
14	2	B	1.77	1.63		1.50
		Average	1.79	1.65		1.53
15	3	A	1.76	1.56		1.50
16	3	B	1.62	1.43		1.42
		Average	1.69	1.50		1.46
17	4	A	1.64	1.56		1.50
18	4	B	1.62	1.56		1.50
		Average	1.63	1.56		1.50
		Average (13–18)	1.70	1.57		1.50

By reference to table 5 it will be seen that, in the early stages of development, the percentages of the fat (ether extract) determined on the fresh-weight basis show no consistent relation to the grades (sizes) of the pea samples. This is probably due to differences in maturity of peas even of the same size in different plots. In the harvest of June 2, 1930, the larger sizes show, with one exception, a higher fat content than the next lower size. The same is true of the harvest of June 10, 1931. These differences are often quite small and of doubtful importance, but they are consistent.

It is evident from table 5 that peas of the same grade but harvested at a later date have, in general, a higher fat content than peas harvested earlier. Generally speaking, the percentages of ether extract parallel the percentages of ash, this being true not only of the grades but also of the age of the corresponding pea samples.

For reasons not known the fat content of the samples of grade 2 peas from the duplicate potash plot (samples 1 and 2) and the sample of one phosphorus plot (sample 2) were higher for the May 28 harvest than for the June 2 harvest. However, these 3 exceptions among 23 comparisons should not invalidate the generalization just drawn.

If the percentages of ether extract are calculated upon a dry-weight basis, all the above relationships are generally reversed, as table 5 shows.

The mean fat content of the three grades of peas from the untreated, the phosphorus-treated, and the nitrogen-treated plots for 1930 are remarkably similar for each harvest and for the two harvests combined. Appreciable differences occur between corresponding samples, but they are somewhat inconsistent. The fat content of the samples from the potash-treated plots was higher than the others in 9 out of 11 instances in 1930, but in 1931 it was lower in all 6 instances than the samples from the untreated plots, resulting in no significant [3] difference for the 2 years. In 1931 the fat content of the nitrogen-treated plots was significantly lower than the check and potash-treated plots, but the differences were quite small and unimportant. Although of doubtful consequence from a practical or culinary standpoint, this fact is interesting in a way that will be discussed later. Upon a dry-weight basis these differences are less striking than when considered as percentages of fresh weight. In 1931 the relationships just discussed for 1930 did not hold. Thus it is not possible to attach any importance to the higher fat content of the potash plots in 1930.

In practically all cases the ether extracts were found to contain not only fat but also pigments, free fatty acids, lecithin, and apparently other constituents as well. The following facts substantiate this statement. All the ether extracts were more or less colored, the color ranging from very light green or light yellow to dark yellowish green. As a rule the ether extract, at least the greater portion of it, was readily soluble in alcohol, even at room temperature. Blue litmus paper dipped into such an alcoholic solution did not change color, but when it was subsequently wet with water the color immediately changed to red. The same reaction is shown by the isolated higher fatty acids. A few crystals of chemically pure palmitic or stearic acid were dissolved in alcohol and blue litmus paper was immersed in the solution. No change of color took place; but if the litmus

[3] As used in the discussion of results in this paper, the term "significant" or "significance" refers to differences between means for which "odds of significance" are greater than 35 to 1, by the method of Student (*1*)

paper was then dipped in water, the blue color changed to red. This reaction, showing the presence of free fatty acids, was obtained with all the extracts of the various grades of peas.

Several ether extracts of the Alaska pea, after being weighed, were taken up with ether, and the ether evaporated on the water bath. The residue was boiled with barium hydroxide for about 2 hours, after which the barium salts of the fatty acids were filtered off and the filtrate evaporated on the water bath at low temperature. The residue was taken up with warm absolute alcohol, in order to remove any choline present, and the whole filtered. The residue on the filter showed the following properties: It was soluble in water, insoluble in absolute alcohol; it contained some barium and, on oxidation with a mixture of potassium nitrate and sodium carbonate, gave a slight reaction for phosphoric acid. These facts point to the presence of lecithin in the ether extracts.

While the ether extracts of the various pea samples were found to contain, in addition to glycerides, also pigments, free fatty acids, and lecithin, they differed from one another both quantitatively and qualitatively. In other words, the pea samples contained not only unequal proportions of ether extracts but they differed also in shade and intensity of color, strength of acidity, solubility, etc. For instance, unlike most of the ether extracts, two of them solidified, at least in part, and were with difficulty soluble in alcohol. The solidified substance may have consisted of wax or a waxlike matter whose nature, however, was not studied. The occurrence of waxlike substances in peas has previously been reported by Schulze and his collaborators (*25*). It is not unreasonable to assume that a careful study of the various constituents of the ether extract may throw additional light on the quality of the Alaska pea.

CARBOHYDRATES

Two grams of ground peas was transferred to a paper extraction thimble which was then covered with glass wool to prevent the substance from being thrown out of the thimble and to insure good uniform extraction. This was effected in a Soxhlet extraction apparatus by means of 60-percent alcohol, which was added to the extraction flask in a quantity sufficient to fill the extractor to the top of the siphon (usually 220 cc), leaving at the same time enough alcohol in the flask to prevent caramelization of the sugars by heat incidental to the extraction. Extraction was continued for at least 4 hours. At the expiration of this time the extract was, with the aid of hot water, transferred quantitatively to an evaporating dish and the alcohol driven off on the water bath. The alcohol-free extract was then transferred by means of hot water to a 250-cc volumetric flask, about 1 cc of 20-percent neutral lead acetate solution added for clarification, made up to the mark, and filtered until perfectly clear.

Estimation of Reducing Sugars

The method employed for the estimation of reducing sugars was that of Bertrand (*2, 8, 9, 21, 22*). Ordinarily a 100-cc portion of the clear filtrate was taken for this estimation. The solution was transferred to an Erlenmeyer flask of about 200- to 250-cc capacity, 20 cc of Bertrand's solution A (copper sulphate) and 20 cc of Bertrand's solution B (Rochelle salt) added, the whole brought to a boil, and

the boiling continued for 3 minutes. The flask was then removed from the flame and the cuprous oxide precipitate allowed to settle well, when the supernatant blue liquid was decanted through a Soxhlet asbestos filter and sucked off. The cuprous oxide precipitate, both in the flask and on the filter, was then washed repeatedly with hot distilled water, after which the filter was removed from the suction flask, and the latter was carefully washed with water to remove any copper present. About 20 cc of Bertrand's solution C (ferric sulphate) was placed in the Erlenmeyer flask, and this dissolved all the cuprous oxide present. This solution was then poured on the Soxhlet filter, placed on the suction flask, and sucked through slowly to dissolve any cuprous oxide present on the filter. The flask was then washed with water, the wash water being used also to wash the Soxhlet filter in order to get quantitatively all the copper solution into the suction flask. This solution was then titrated with Bertrand's solution D (standard potassium permanganate) to a pink color. The results were calculated as glucose.

The potassium permanganate solution was standardized against ammonium oxalate.

Determination of Total Sugars

For the determination of total sugars 75 or 100 cc of the solution, as employed for the estimation of reducing sugars, was used. The inversion was effected by the method of Herzfeld (*7*, *10*). The solution was transferred to a 100-cc volumetric flask, 8 cc of 36-percent hydrochloric acid was added, and the whole shaken carefully. The flask with its contents was heated to 67° to 70° C. by placing it up to the neck in a water bath at 70°, this temperature being maintained for 5 more minutes. Total inversion was never allowed to last more than 10 minutes. At the expiration of that time the flask was cooled with cold water, the hydrolysate neutralized with sodium hydroxide solution and made up to 100 cc. This was divided into two equal portions and employed for the determination of total sugars according to Bertrand's method as already outlined. The results were calculated as invert sugar.

Estimation of Sucrose

Sucrose was calculated by subtracting the percentage of reducing sugars from that of total sugars and multiplying the difference by the factor 0.95.

Estimation of Starch

Strictly speaking, the estimation of starch deals with total acid-hydrolyzable substances rather than with starch alone, since the pea is known to contain, in addition to starch, other acid-hydrolyzable polysaccharides such as sucrose, cellulose, dextrin, hemicelluloses (xylan, araban, galactan, and mannan), and perhaps other polysaccharides and substances that yield some glucose upon hydrolysis. In this connection the following facts should be borne in mind: Sucrose has been quantitatively removed from the peas by extraction with 60-percent alcohol prior to hydrolysis; cellulose is insoluble in 2-percent hydrochloric acid used for hydrolysis of the polysaccharides; dextrin is known to occur in the pea to the extent of but 6 percent and may have been wholly or partly extracted by the 60-percent

alcohol, while the exact nature and quantities of all hemicelluloses occurring in peas are not very well known. It cannot be doubted that the hemicelluloses are hydrolyzed, at least in part, under the conditions of hydrolysis as employed in this investigation. However, it seems fairly safe to state that the changes of total hydrolyzable substances give a good index to the changes in starch content. For this reason the acid-hydrolyzable polysaccharides are referred to here as starch.

The starch was estimated essentially according to the method described by Lohrisch (*17, p. 375*), Zemplén (*27*), and Schmidt (*24, p. 924*). The residue that remained from the 2 g of peas, after the sugars had been extracted with 60-percent alcohol, was transferred to a 1-l [4] flask, to which 150 cc of distilled water and 8 cc of 36-percent hydrochloric acid were added, and the mixture was hydrolyzed by boiling gently for 2 hours. The hydrolysate was then cooled, neutralized with sodium hydroxide solution, transferred to a 250-cc volumetric flask, made up to volume with water, and filtered clear. Ten-cubic-centimeter portions of this solution were used for estimating the sugar as glucose according to Bertrand's method. The starch was calculated by multiplying the glucose found by the factor 0.9. The results obtained with the various carbohydrates are summarized in tables 6, 7, and 8.

TABLE 6.—*Percentage of reducing sugars in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Reducing sugars from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.039	0.048	0.039	0.079
2	2	B	.034	.042	.041	.080
		Average	.036	.045	.040	.079
3	3	A	.064	.045	.033	.061
4	3	B	.045	.070	.037	.075
		Average	.054	.057	.035	.068
5	4	A	.048		.031	.064
6	4	B	.036	.058	.030	.054
		Average	.042	.058	.030	.059
		Average (1–6)	.044	.053	.035	.069
Harvest of June 2:						
7	2	A	.071	.056	.048	.082
8	2	B	.029	.037	.044	.060
		Average	.050	.046	.046	.071
9	3	A	.042	.033	.078	.096
10	3	B	.037	.081	.047	.088
		Average	.039	.057	.062	.092
11	4	A	.050	.050	.048	.100
12	4	B	.034	.093	.041	.048
		Average	.042	.071	.044	.074
		Average (7–12)	.043	.058	.051	.079
		Average (1–12)	.044	.056	.043	.074

[4] A large flask is indispensable, otherwise loss of liquid may occur at the beginning of boiling, when the liquid foams quite badly.

TABLE 6.—*Percentage of reducing sugars in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Continued

1931 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Reducing sugars from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:			*Percent*	*Percent*	*Percent*	*Percent*
13	2	A	0.036	0.043		0.074
14	2	B	.042	.034		.084
		Average	.039	.039		.073
15	3	A	.040	.039		.057
16	3	B	.053	.060		.053
		Average	.047	.050		.055
17	4	A	.042	.059		.113
18	4	B	.059	.061		.040
		Average	.051	.060		.077
		Average (13–18)	.045	.049		.070

1930 CROP, OVEN-DRY WEIGHT

Harvest of May 28:						
1	2	A	0.19	0.23	0.19	0.38
2	2	B	.16	.20	.19	.38
		Average	.18	.22	.19	.38
3	3	A	.28	.19	.15	.28
4	3	B	.19	.30	.16	.33
		Average	.24	.25	.16	.31
5	4	A	.20		.13	.28
6	4	B	.15	.23	.12	.23
		Average	.18	.23	.13	.26
		Average (1–6)	.20	.23	.16	.31
Harvest of June 2:						
7	2	A	.30	.23	.19	.36
8	2	B	.12	.15	.17	.24
		Average	.21	.19	.18	.30
9	3	A	.15	.12	.28	.38
10	3	B	.13	.29	.16	.31
		Average	.14	.21	.22	.35
11	4	A	.17	.17	.16	.34
12	4	B	.11	.31	.13	.16
		Average	.14	.24	.15	.25
		Average (7–12)	.16	.21	.18	.30
		Average (1–12)	.18	.22	.17	.31

1931 CROP, OVEN-DRY WEIGHT

Harvest of June 10:						
13	2	A	0.17	0.20		0.33
14	2	B	.19	.15		.38
		Average	.18	.18		.36
15	3	A	.15	.15		.21
16	3	B	.20	.22		.20
		Average	.18	.19		.21
17	4	A	.14	.20		.37
18	4	B	.20	.20		.13
		Average	.17	.20		.25
		Average (13–18)	.18	.19		.27

TABLE 7.—*Percentage of sucrose in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Sucrose from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	4.22	3.62	3.98	4.59
2	2	B	3.55	3.61	3.89	3.93
		Average	3.88	3.61	3.93	4.26
3	3	A	3.35	2.83	3.74	4.03
4	3	B	2.72	3.03	3.31	3.53
		Average	3.03	2.93	3.52	3.78
5	4	A	3.46		3.28	3.47
6	4	B	2.53	2.88	2.87	3.02
		Average	2.99	2.88	3.07	3.24
		Average (1–6)	3.30	3.19	3.51	3.76
Harvest of June 2:						
7	2	A	4.14	3.16	3.62	4.20
8	2	B	2.90	3.83	3.29	3.57
		Average	3.52	3.49	3.45	3.88
9	3	A	2.65	2.82	3.28	3.27
10	3	B	2.41	3.13	2.94	2.55
		Average	2.53	2.97	3.11	2.91
11	4	A	2.38	2.38	2.98	3.21
12	4	B	2.43	3.21	2.56	2.57
		Average	2.40	2.79	2.77	2.89
		Average (7–12)	2.82	3.09	3.11	3.23
		Average (1–12)	3.06	3.14	3.31	3.49

1931 CROP, FRESH WEIGHT

Harvest of June 10:						
13	2	A	3.42	4.11		3.86
14	2	B	3.81	3.61		4.23
		Average	3.62	3.86		4.05
15	3	A	3.08	3.08		2.91
16	3	B	2.77	2.98		3.40
		Average	2.93	3.03		3.16
17	4	A	2.54	2.55		2.22
18	4	B	2.99	2.65		3.45
		Average	2.77	2.60		2.84
		Average (13–18)	3.10	3.16		3.35

TABLE 7.—*Percentage of sucrose in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Con.

1930 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Sucrose from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	20.61	17.19	19.18	22.21
2	2	B	16.87	17.14	18.24	18.67
		Average	18.74	17.17	18.71	20.44
3	3	A	14.74	12.01	16.77	18.52
4	3	B	11.60	13.05	14.17	15.44
		Average	13.17	12.53	15.47	16.98
5	4	A	14.48		13.69	15.23
6	4	B	10.51	11.45	11.35	14.04
		Average	12.50	11.45	12.52	14.64
		Average (1-6)	14.80	14.17	15.57	17.35
Harvest of June 2:						
7	2	A	17.39	12.81	14.37	18.43
8	2	B	11.64	15.68	12.81	14.38
		Average	14.52	14.25	13.59	16.41
9	3	A	9.56	10.18	11.76	12.94
10	3	B	8.47	11.17	10.10	8.96
		Average	9.02	10.68	10.93	10.95
11	4	A	8.10	8.07	9.85	10.94
12	4	B	7.78	10.74	8.15	8.65
		Average	7.94	9.41	9.00	9.80
		Average (7-12)	10.49	11.44	11.17	12.38
		Average (1-12)	12.65	12.68	13.37	14.87

1931 CROP, OVEN-DRY WEIGHT

Harvest of June 10:						
13	2	A	15.96	18.90		17.28
14	2	B	17.05	16.02		19.07
		Average	16.51	17.46		18.18
15	3	A	11.58	11.69		10.77
16	3	B	10.35	10.89		12.90
		Average	10.97	11.29		11.84
17	4	A	8.44	8.59		7.27
18	4	B	10.20	8.77		11.29
		Average	9.32	8.68		9.28
		Average (13-18)	12.26	12.48		13.10

TABLE 8.—*Percentage of acid-hydrolyzable substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Acid-hydrolyzable substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	5.82	5.07	5.69	5.00
2	2	B	6.01	6.70	6.22	6.44
		Average	5.91	5.88	5.95	5.72
3	3	A	7.66	8.06	6.90	6.60
4	3	B	8.40	8.16	8.38	7.83
		Average	8.03	8.11	7.59	7.21
5	4	A	8.38	.....	8.03	7.81
6	4	B	8.66	9.84	8.96	8.19
		Average	8.52	9.84	8.49	8.00
		Average (1–6)	7.49	7.56	7.34	6.98
Harvest of June 2:						
7	2	A	8.24	8.31	8.77	6.93
8	2	B	8.92	8.73	8.87	8.14
		Average	8.58	8.52	8.82	7.53
9	3	A	11.07	9.56	10.60	10.10
10	3	B	11.81	11.10	12.05	11.15
		Average	11.44	10.33	11.32	10.62
11	4	A	12.34	11.85	12.48	12.30
12	4	B	13.32	13.60	13.83	11.82
		Average	12.83	12.72	13.15	12.06
		Average (7–12)	10.95	10.53	11.10	10.07
		Average (1–12)	9.22	9.18	9.22	8.52

1931 CROP, FRESH WEIGHT

Harvest of June 10:						
13	2	A	5.97	6.53	.....	7.18
14	2	B	6.60	7.53	.....	6.48
		Average	6.29	7.03	.....	6.83
15	3	A	10.20	9.55	.....	10.20
16	3	B	10.07	10.89	.....	9.30
		Average	10.14	10.22	.....	9.75
17	4	A	12.54	12.67	.....	11.91
18	4	B	11.82	13.19	.....	12.50
		Average	12.18	12.93	.....	12.21
		Average (13–18)	9.53	10.06	.....	9.60

TABLE 8.—*Percentage of acid-hydrolyzable substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Continued

1930 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Acid-hydrolyzable substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	28.49	24.10	27.42	24.19
2	2	B	28.59	31.82	29.13	30.48
		Average	28.54	27.96	28.28	27.34
3	3	A	33.71	34.22	30.94	30.39
4	3	B	35.79	35.17	35.93	34.30
		Average	34.75	34.70	33.44	32.35
5	4	A	35.06		33.52	34.30
6	4	B	35.91	39.13	35.60	34.65
		Average	35.49	39.13	34.56	34.48
		Average (1–6)	32.93	32.89	32.09	31.39
Harvest of June 2:						
7	2	A	34.65	33.97	34.84	30.39
8	2	B	35.82	35.71	34.55	32.77
		Average	35.24	34.84	34.70	31.58
9	3	A	39.99	34.43	38.01	40.00
10	3	B	41.51	39.60	41.45	39.18
		Average	40.75	37.02	39.73	39.59
11	4	A	41.94	40.11	41.21	41.96
12	4	B	42.74	45.45	44.07	39.71
		Average	42.34	42.78	42.64	40.84
		Average (7–12)	39.44	38.21	39.02	37.34
		Average (1–12)	36.18	35.79	35.56	34.36

1931 CROP, OVEN-DRY WEIGHT

Harvest of June 10:						
13	2	A	27.86	30.06		32.16
14	2	B	29.54	33.38		29.20
		Average	28.70	31.72		30.68
15	3	A	38.29	36.28		37.78
16	3	B	37.67	39.76		35.22
		Average	37.98	38.02		36.50
17	4	A	41.68	42.61		38.98
18	4	B	40.26	43.59		40.88
		Average	40.97	43.10		39.93
		Average (13–18)	35.88	37.61		35.70

DISCUSSION AND INTERPRETATION OF CARBOHYDRATE RESULTS

From table 6 it will be seen that, as a rule, the proportions of reducing sugars are almost negligible, ranging from 0.03 to 0.10 percent in the 1930 peas, and from 0.036 to 0.113 percent in the 1931 peas, calculated on the basis of fresh weight. There appears to be some difference in the percentage of reducing sugars obtained from peas grown on the variously treated plots. Thus, in the case of the 1930

peas raised in untreated soil the reducing sugars ranged from 0.029 to 0.071 percent, these extremes being duplicates; the average of all samples was 0.044 percent. Very similar results were obtained with the peas grown in phosphorus-treated soil, in which the percentage of reducing sugar ranged from 0.03 to 0.078, the average being 0.043 percent. Somewhat larger was the proportion of reducing sugars in peas from potash-treated soil, in which the percentage ranged from 0.033 to 0.093, with an average of 0.056 percent in 1930. However, the differences between corresponding samples were so inconsistent that no significance can be attached to the differences between the means just referred to. In peas from the nitrogen-treated plot the proportions of reducing sugars were slightly and significantly larger than from the check or those receiving other treatments. The analytical averages of the 1931 peas are similar to those of the 1930 peas. Considering the fact that the percentage of reducing sugars is extremely small, averaging 0.044 and 0.045 percent in peas from the untreated plot for 1930 and 1931, respectively, it would appear that there is some physiological significance in the higher percentage of reducing sugars in peas from the nitrogen-treated plot (average, 0.074 and 0.070 percent for 1930 and 1931, respectively) as compared with that in the peas from the other plots. This higher content of reducing substances, although significant statistically, is of such small magnitude as to be of no practical importance. The foregoing observations concerning determinations made on the fresh-weight basis apply equally to those made on the oven-dry weight basis.

Table 7 reveals distinct regularities in the proportions of sucrose. The figures for total sugars have been omitted, since sucrose constitutes 96 to 99 percent of the total sugars and the reducing sugars are negligible. The large-sized peas had a smaller percentage of sucrose than the small-sized peas, regardless of the time of harvest or the treatment of the plot. The older peas had a smaller sucrose content than the younger ones of the same size, age and sucrose content standing in reverse ratio.

The mean percentages of sucrose in the peas from the untreated and potash-treated plots were practically the same. The sucrose percentage in peas that received the phosphorus treatment was significantly higher than that of the check, but not significantly different from that of peas from the other plots.

The regularities in sucrose content of the peas on the fresh-weight basis hold good also for that of peas on the dry-weight basis, but the differences resulting from age and size are more striking, as will be seen by reference to table 7. That the fertilizers did not have a greater influence on the sucrose content may have been due in part to the fact that from the outset the plots were fairly fertile. At the same time it is recognized that in general it is difficult to alter the composition of seeds by these means.

Table 8 (fresh-weight basis) shows that the peas of larger grades had a correspondingly higher starch content than the peas of smaller grades, this being true of peas raised in fertilized and unfertilized plots. In other words, the starch content of the peas varied directly with their size. Another regularity noted was the higher percentage of starch in peas of the later harvest. The regularities observed in the starch content of the peas when the determinations were made on

the fresh-weight basis also hold true when the starch content was determined on the oven-dry weight basis (table 8).

With regard to the influence of the various fertilizers on the starch content of the peas, table 8 shows that the 1930 peas from the untreated plot had on an average 9.22 percent of starch, those from the potash-treated plot had on an average 9.18 percent, while the average starch content of the peas from the plots treated with phosphorus and nitrogen was, respectively, 9.22 and 8.52 percent. By reducing these figures to the starch content of the peas from the untreated plot taken as 100, it will be found that the starch content of the peas from the plots treated with potash, phosphorus, and nitrogen is, respectively, 99.5, 100.0, and 92.4 percent. Thus, the nitrogen treatment has here the only significant influence on the starch formation, the effect being characteristic of delayed maturity. The difference is significant with reference to the potash- and phosphorus-treated plots as well as to the untreated plots.

The facts pointed out in the foregoing discussion will be better comprehended when the carbohydrate metabolism in peas is taken into consideration. Let us assume, as is generally assumed for plants, that the carbohydrate metabolism in peas takes place according to the following equations:

(1) $\underset{\text{Carbon dioxide}}{(CO_2} + \underset{\text{Water}}{H_2O)} \longrightarrow \underset{\text{Formaldehyde}}{CH_2O} \longrightarrow \underset{\text{Glucose}}{C_6H_{12}O_6}$

(2) $\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6} - \underset{\text{Water}}{H_2O} = \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}}$

(3) $m(\underset{\text{Glucose}}{C_6H_{12}O_6}) - n(\underset{\text{Water}}{H_2O}) = (\underset{\text{Starch}}{C_6H_{10}O_5})X$

Then the simplest interpretation of the results obtained is that the first sugar to appear, glucose (equation 1), is rapidly changing to sucrose (equation 2). It is for this reason that the proportion of reducing sugars in the peas is quite insignificant. However, it appears that the condensation of reducing substances to sucrose and finally to starch, which is characteristic of maturity, was somewhat delayed in the peas grown on nitrogen-treated soil. This is evident from the fact that the reducing sugars in the 1930 peas from the nitrogen-treated plot (average 0.074) are significantly greater than those in peas from any of the other plots (average 0.043 to 0.056). That the nitrogen has a somewhat retarding influence on the rate of maturity of the peas is also evident from the sucrose content of the peas (table 7). The average sucrose content of the 1930 peas from the nitrogen-treated plot, the check, and the plots treated with potash and phosphorus is, respectively, 3.49, 3.06, 3.14, and 3.31. The phosphorus-treated plot produced peas of a very slightly but significantly higher sucrose content than did the check and potash-treated plots, but inasmuch as peas from the plot given the phosphorus treatment did not exhibit other differences consistently characteristic of either delayed or hastened maturity, this point is in itself unimportant. Thus, only the application of nitrogen had a definite, retarding influence on the rate of maturity of the peas, as evidenced by the sugar content. The sucrose content of the peas harvested on June 2 was smaller than that of the peas harvested on May 28 and is in full harmony with the stated metabolism in the peas.

In the later stages of development glucose is changed chiefly to starch (equation 3). For this reason the more mature peas must necessarily have a larger percentage of starch and a correspondingly smaller percentage of sucrose. That this is actually the case a glance at tables 8 and 9 will show. The fairly high percentage of sucrose and starch in peas grown in the variously treated plots is an indication that these two carbohydrates represent important reserve materials of the peas.

TOTAL NITROGEN

The total nitrogen was determined by Gunning's modification of the Kjeldahl method.

Table 9 shows that when determinations were made on a fresh-weight basis the small-sized peas as a rule had a lower percentage of nitrogen than the large-sized, and that peas harvested early had a lower nitrogen content than those harvested later. On the other hand, when determinations were made on a dry-weight basis the nitrogen content ordinarily decreased with increasing size and age of the peas.

TABLE 9.—*Percentage of total nitrogen in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.943	1.013	0.956	0.986
2	2	B	1.004	.967	.935	1.011
		Average	.973	.990	.945	.998
3	3	A	1.003	1.084	1.015	1.033
4	3	B	1.091	1.033	1.055	1.078
		Average	1.047	1.058	1.035	1.055
5	4	A	1.022		1.054	1.041
6	4	B	1.106	1.127	1.110	1.059
		Average	1.064	1.127	1.082	1.050
		Average (1–6)	1.028	1.045	1.031	1.034
Harvest of June 2:						
7	2	A	1.010	1.092	1.102	1.052
8	2	B	1.126	1.029	1.071	1.150
		Average	1.068	1.060	1.086	1.101
9	3	A	1.169	1.235	1.185	1.119
10	3	B	1.246	1.184	1.200	1.286
		Average	1.207	1.209	1.192	1.202
11	4	A	1.215	1.329	1.286	1.296
12	4	B	1.346	1.234	1.296	1.330
		Average	1.280	1.281	1.291	1.313
		Average (7–12)	1.185	1.184	1.190	1.205
		Average (1–12)	1.106	1.121	1.105	1.119

TABLE 9.—*Percentage of total nitrogen in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Continued

1931 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated: Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:			*Percent*	*Percent*	*Percent*	*Percent*
13	2	A	0.940	0.952		0.990
14	2	B	.991	.981		.957
		Average	.966	.967		.974
15	3	A	1.156	1.126		1.156
16	3	B	1.157	1.143		1.111
		Average	1.157	1.135		1.134
17	4	A	1.299	1.285		1.302
18	4	B	1.245	1.274		1.272
		Average	1.272	1.280		1.287
		Average (13–18)	1.131	1.127		1.131

1930 CROP, OVEN-DRY WEIGHT

Harvest of May 28:						
1	2	A	4.61	4.81	4.61	4.77
2	2	B	4.77	4.50	4.67	4.81
		Average	4.69	4.70	4.64	4.79
3	3	A	4.41	4.60	4.55	4.75
4	3	B	4.65	4.45	4.52	4.72
		Average	4.53	4.53	4.54	4.74
5	4	A	4.27		4.40	4.57
6	4	B	4.59	4.48	4.41	4.58
		Average	4.43	4.48	4.41	4.58
		Average (1–6)	4.55	4.59	4.53	4.70
Harvest of June 2:						
7	2	A	4.25	4.46	4.38	4.61
8	2	B	4.52	4.21	4.17	4.63
		Average	4.39	4.34	4.28	4.62
9	3	A	4.22	4.45	4.25	4.43
10	3	B	4.38	4.22	4.13	4.52
		Average	4.30	4.34	4.19	4.48
11	4	A	4.13	4.50	4.25	4.42
12	4	B	4.32	4.12	4.13	4.47
		Average	4.23	4.31	4.19	4.45
		Average (7–12)	4.30	4.33	4.22	4.51
		Average (1–12)	4.43	4.44	4.37	4.61

1931 CROP, OVEN-DRY WEIGHT

Harvest of June 10:						
13	2	A	4.39	4.38		4.43
14	2	B	4.44	4.35		4.31
		Average	4.42	4.37		4.37
15	3	A	4.34	4.28		4.28
16	3	B	4.33	4.17		4.21
		Average	4.34	4.23		4.25
17	4	A	4.32	4.32		4.26
18	4	B	4.24	4.21		4.16
		Average	4.28	4.27		4.21
		Average (13–18)	4.34	4.29		4.28

There were no significant differences in the total nitrogen content of the peas from plots receiving different treatments, which is rather surprising in view of the rather marked increase of sucrose and decrease of starch in peas from the nitrogen plot.

PROTEIN AND NONPROTEIN NITROGEN

The estimation of the protein nitrogen was made according to Stutzer's method (*26*) as applied by one of the writers and reported in previous publications (*12, 13, 14, 15*). The estimation of the nonprotein nitrogen was either made directly by ascertaining the nitrogen in the filtrate from the protein precipitate as obtained by means of Stutzer's copper solution (*11, 16, 26*) or calculated by difference from 100 (tables 10 and 11).

Table 10 shows that the peas of smaller size had a smaller protein nitrogen content than those of larger size.

What is true of the relationship between the different sizes of peas is also true of the relationship between peas of different ages regardless of treatment. For instance, in 1930 grades 2, 3, and 4 of peas from the untreated plot harvested May 28 have 41.21, 48.53, and 51.29 percent of protein nitrogen, respectively (samples 1, 3, and 5), while the corresponding grades of peas harvested June 2 have higher proportions of protein nitrogen. The same holds true of the peas from the potash-, nitrogen-, and phosphorus-treated plots. The nonprotein nitrogen of peas from the various plots stands in reverse ratio to the protein nitrogen, as would be expected. Exactly the same relationships regarding size and age of the peas hold true when the percentages of protein and nonprotein nitrogen are calculated on the dry-weight basis.

The regularities herein reported regarding the decreasing sucrose content and the increasing starch and protein content with greater size and age of the peas are in harmony with the findings of other investigators (*3, 4, 23*). These facts will be better comprehended if the anabolic processes taking place in the pea are taken into account. The nitrates taken up by the pea from the soil are successively converted into nitrites, ammonia, and then into the various organic compounds according to the following scheme:

Nitrates→ nitrites→ ammonia→ mono- and di-amino acids→ acid amides→ polypeptides (peptones, proteoses) (*6, pp. 23–53*)→ proteins.

From this scheme it is plainly evident that in the earlier stages the nonproteins (amino acids, acid amides) are dominant, whereas in the latter stages the proteins, namely, legumin, vicilin, legumelin, and proteose (*18, 19, 20*) are dominant. It would seem that the regularities so conspicuously displayed by the protein nitrogen of peas from the variously treated plots, especially the very considerable and consistent differences in the protein nitrogen between peas of different sizes and of different ages, make this estimation an accurate means of determining the stage of maturity of the Alaska pea.

Although there are no significant differences in the total nitrogen content of the peas from the variously treated plots, some consistent differences occur in the distribution of the nitrogen. The mean percentage of protein nitrogen based on fresh weight of peas from the various plots for 1930 was: Check, 0.614; potash, 0.644; phosphorus, 0.627; and nitrogen, 0.599. The corresponding percentages of nonprotein nitrogen were 0.492, 0.477, 0.485, and 0.520. The differences in amount of these constituents calculated on a fresh-weight basis were too small and too variable to appear important. However, if the percentage of protein or nonprotein nitrogen is calculated on the basis of total nitrogen, some very significant differences between plots are evident. The peas from the potash plot show a protein-nitrogen content that is 56.73 percent of the total, or 1.3 times the nonprotein portion. Calculated similarly, those from the check and nitrogen-treated plots show a protein content of 54.61 and 52.66 percent, or 1.2 and 1.1 times the nonprotein-nitrogen content. The increased proportion of protein nitrogen of the potash-treated peas is not statistically significant, but the decreased proportion of the nitrogen-treated peas is significant when compared with the check or the other treatments. The peas from the phosphorus-treated plot also show a significantly higher proportion of protein nitrogen than do those of the check.

Despite the statistical significance of the differences here discussed, it is doubtful whether the magnitudes are such as to be of great practical importance from the standpoint of culinary quality. The data show, however, a tendency for potash treatment to hasten certain processes that are characteristic of maturity, and for nitrogen treatment to delay those processes.

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TABLE 10.—*Percentage of protein and nonprotein nitrogen of the Alaska pea on fresh-weight basis and percentage of protein nitrogen based on total nitrogen, 1930 and 1931*

1930 CROP

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated											
			Check			Potash			Phosphoric acid			Nitrogen		
			Protein nitrogen		Nonprotein nitrogen	Protein nitrogen		Nonprotein nitrogen	Protein nitrogen		Nonprotein nitrogen	Protein nitrogen		Nonprotein nitrogen
			Fresh-weight basis	Total nitrogen basis	Fresh-weight basis	Fresh-weight basis	Total nitrogen basis	Fresh-weight basis	Fresh-weight basis	Total nitrogen basis	Fresh-weight basis	Fresh-weight basis	Total nitrogen basis	Fresh-weight basis
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.389	41.21	0.554	0.431	40.75	0.600	0.407	42.52	0.549	0.391	39.62	0.595
2	2	B	.431	42.98	.573	.438	45.32	.529	.399	42.61	.536	.437	43.24	.574
		Average	.410	42.09	.563	.435	43.03	.564	.403	42.56	.542	.414	41.43	.584
3	3	A	.487	48.53	.516	.561	51.74	.523	.498	49.01	.517	.452	43.79	.581
4	3	B	.552	50.54	.539	.553	53.48	.480	.532	50.44	.523	.528	48.94	.550
		Average	.519	49.53	.527	.557	52.61	.501	.515	49.72	.520	.490	46.36	.565
5	4	A	.524	51.29	.498				.558	52.95	.496	.516	49.45	.525
6	4	B	.596	53.81	.510	.674	59.82	.453	.619	55.78	.491	.564	53.28	.495
		Average	.560	52.55	.504	.674	59.82	.453	.588	54.36	.493	.540	51.36	.510
		Average (1–6)	.496	48.06	.531	.531	50.22	.517	.502	48.88	.518	.481	46.38	.553
Harvest of June 2:														
7	2	A	.523	51.76	.487	.575	52.69	.517	.591	53.65	.511	.493	46.85	.559
8	2	B	.603	53.54	.523	.572	55.58	.457	.599	55.88	.472	.604	52.48	.546
		Average	.563	52.65	.505	.573	54.13	.487	.595	54.76	.491	.548	49.66	.552
9	3	A	.706	60.43	.463	.785	63.60	.450	.731	61.65	.454	.669	59.82	.450
10	3	B	.802	64.38	.444	.774	65.40	.410	.799	66.59	.501	.806	62.61	.480
		Average	.754	62.40	.453	.779	64.50	.430	.765	64.12	.477	.737	61.21	.465

11	4	A	.806	66.34	.409	.880	66.22	.449	.881	68.24	.405	.851	65.61	.445
12	4	B	.951	70.60	.395	.857	69.42	.377	.917	70.70	.379	.881	66.22	.449
		Average	.878	68.47	.402	.868	67.82	.413	.899	69.47	.392	.866	65.91	.447
		Average (7-12)	.732	61.17	.453	.740	62.15	.443	.753	62.78	.453	.717	58.94	.488
		Average (1-12)	.614	54.61	.492	.644	56.73	.477	.627	55.83	.485	.599	52.66	.520

1931 CROP

Harvest of June 10:														
13	2	A	0.446	47.38	0.495	0.448	47.03	0.504				0.474	47.86	0.516
14	2	B	.476	47.97	.516	.489	49.89	.492				.444	46.40	.513
		Average	.461	47.68	.506	.469	48.46	.498				.459	47.13	.515
15	3	A	.719	62.21	.437	.687	60.98	.439				.689	59.58	.467
16	3	B	.724	62.59	.433	.726	63.55	.416				.657	59.14	.454
		Average	.722	62.40	.435	.707	62.27	.428				.673	59.36	.461
17	4	A	.899	69.21	.400	.880	68.52	.404				.892	68.54	.409
18	4	B	.866	69.58	.379	.908	71.26	.366				.859	67.55	.411
		Average	.883	69.40	.390	.894	69.89	.385				.876	68.05	.413
		Average (13-18)	.688	59.82	.443	.690	60.21	.437				.669	58.18	.462

TABLE 11.—*Percentage of protein and nonprotein nitrogen of the Alaska pea on oven-dry weight basis and percentage of nonprotein nitrogen based on total nitrogen*

1930 CROP

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated											
			Check			Potash			Phosphoric acid			Nitrogen		
			Protein nitrogen	Nonprotein nitrogen		Protein nitrogen	Nonprotein nitrogen		Protein nitrogen	Nonprotein nitrogen		Protein nitrogen	Nonprotein nitrogen	
			Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis	Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis	Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis	Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	1.90	2.71	58.79	1.96	2.85	59.25	1.96	2.65	57.48	1.89	2.88	60.38
2	2	B	2.05	2.72	57.02	2.08	2.51	54.68	1.99	2.68	57.39	2.08	2.73	56.76
		Average	1.98	2.72	57.91	2.02	2.68	56.97	1.98	2.67	57.44	1.99	2.81	58.57
3	3	A	2.14	2.27	51.47	2.38	2.22	48.26	2.23	2.32	50.99	2.08	2.67	56.21
4	3	B	2.35	2.30	49.46	2.38	2.07	46.52	2.28	2.24	49.56	2.31	2.41	51.06
		Average	2.25	2.29	50.47	2.38	2.15	47.39	2.26	2.28	50.28	2.20	2.54	53.64
5	4	A	2.19	2.08	48.71				2.33	2.07	47.05	2.26	2.31	50.55
6	4	B	2.47	2.12	46.19	2.68	1.80	40.18	2.46	1.95	44.22	2.44	2.14	46.72
		Average	2.33	2.10	47.45	2.68	1.80	40.18	2.40	2.01	45.64	2.35	2.23	48.64
		Average (1–6)	2.18	2.37	51.94	2.30	2.29	49.78	2.21	2.32	51.12	2.18	2.52	53.61
Harvest of June 2:														
7	2	A	2.20	2.05	48.24	2.35	2.11	47.31	2.35	2.03	46.35	2.16	2.45	53.15
8	2	B	2.42	2.10	46.46	2.34	1.87	44.42	2.33	1.84	44.12	2.43	2.20	47.52
		Average	2.31	2.08	47.35	2.35	1.99	45.87	2.34	1.94	45.24	2.30	3.33	50.34
9	3	A	2.55	1.67	39.57	2.83	1.62	36.40	2.62	1.63	38.35	2.65	1.78	40.18
10	3	B	2.82	1.56	35.62	2.76	1.46	34.60	2.75	1.38	33.41	2.83	1.69	37.39
		Average	2.69	1.62	37.60	2.80	1.54	35.50	2.69	1.51	35.88	2.74	1.74	38.79

11	4	A	2.74	1.39	33.66	2.98	1.52	33.78	2.90	1.35	31.76	2.90	1.52	34.39
12	4	B	3.05	1.27	29.40	2.86	1.26	30.58	2.92	1.21	29.30	2.96	1.51	33.78
		Average	2.90	1.33	31.53	2.92	1.39	32.18	2.91	1.28	30.53	2.93	1.52	34.09
		Average (7–12)	2.63	1.67	38.83	2.69	1.64	37.85	2.65	1.57	37.22	2.66	1.86	41.07
		Average (1–12)	2.41	2.02	45.38	2.51	1.94	43.27	2.43	1.95	44.17	2.42	2.19	47.34
		1931 CROP												
Harvest of June 10:														
13	2	A	2.08	2.31	52.62	2.06	2.32	52.97				2.12	2.31	52.14
14	2	B	2.13	2.31	52.03	2.17	2.18	50.11				2.00	2.31	53.60
		Average	2.11	2.31	52.33	2.12	2.25	51.54				2.06	2.31	52.87
15	3	A	2.70	1.64	37.79	2.61	1.67	39.02				2.55	1.73	40.42
16	3	B	2.71	1.62	37.41	2.65	1.52	36.45				2.49	1.72	40.86
		Average	2.71	1.63	37.60	2.63	1.60	37.74				2.52	1.73	40.64
17	4	A	2.99	1.33	30.79	2.96	1.36	31.48				2.92	1.34	31.46
18	4	B	2.95	1.29	30.42	3.00	1.21	28.74				2.81	1.35	32.45
		Average	2.97	1.31	30.61	2.98	1.29	30.11				2.87	1.35	31.96
		Average (13–18)	2.59	1.75	40.18	2.58	1.71	39.80				2.48	1.79	41.82

SUMMARY AND CONCLUSIONS

The application of superphosphate at the rate of 1,000 pounds per acre (160 pounds phosphoric acid) produced a definite increase in yield of the Alaska pea over the untreated plot, amounting to 24 and 18 percent (fresh weight) for the seasons of 1930 and 1931, respectively. On the other hand, the application of 300 pounds of muriate of potash per acre (144 pounds potash) and of 400 pounds nitrate of soda plus 280 pounds of sulphate of ammonia (116 pounds nitrogen) gave differences in yield, as compared with the untreated plot, which were neither sufficiently great nor sufficiently consistent to permit the drawing of definite conclusions.

Records of direct observations in the field, as well as the very similar maturity indices for the peas from the treated and untreated plots, do not show any perceptible consistent differences in the rate of maturity. This is in harmony with earlier field observations and with reports of other investigators as well as with the chemical analyses of the peas from plots treated with potash and phosphorus. However, the peas from the nitrogen-treated plot showed, on an average for the entire season, a somewhat higher percentage of reducing sugars, a significantly higher percentage of sucrose, and a distinctly lower starch content as compared with the peas from the untreated plot.

Although the various treatments were without consistent effect on content of total nitrogen, the peas of the nitrogen-treated plot exhibited a slightly but significantly smaller proportion of protein nitrogen than peas from the check or any of the other plots. On the other hand, peas grown on the potash-treated and phosphorus-treated plots showed a larger proportion of protein than did the check, suggesting more advanced or hastened maturity as a result of the treatments. However, the phosphorus treatment was followed by no noticeable difference in percentage of any of the carbohydrates, of ash, or of ether extract. Thus, it seems safe to conclude that the maturity and hence the quality of the peas were unaffected by the superphosphate. The peas from the potash plots, on the other hand, exhibited slightly and significantly higher ash and ether-extract content than did the checks and those receiving the other treatments. Potash treatment was accompanied by increases in 3 of 5 constituents which are known to increase with maturity. Only the two carbohydrate fractions, sucrose and starch, which are believed to be of primary importance in determining quality and maturity, were unaffected. Thus it seems that potash does have a slight tendency to hasten the maturity of the pea. This tendency, however, is so very slight, the differences are of such very small magnitude, that they are of questionable practical importance.

Stages of maturity are characterized by distinct and marked differences. In general, more mature peas, i.e., larger peas or peas of the same size but of later harvest, have a larger percentage of ash, of ether extract (fat), total nitrogen, starch, and protein nitrogen, and a lower percentage of sucrose. On a dry-weight basis the percentages of all constituents except starch and protein nitrogen decrease as maturity progresses.

Muriate of potash and superphosphate applied singly are apparently without consistent and significant effect upon the rate of development or the quality of Alaska peas as indicated by partial chemical analysis

or behavior in the field. Readily available nitrogen tends to delay maturity appreciably, as is indicated by the higher sugar and lower protein and hydrolyzable-polysaccharide content.

LITERATURE CITED

(1) ANONYMOUS.
1915–17. MISCELLANEA—TABLES FOR ESTIMATING THE PROBABILITY THAT THE MEAN OF A UNIQUE SAMPLE OF OBSERVATIONS LIES BETWEEN ANY GIVEN DISTANCE OF THE MEAN OF THE POPULATION FROM WHICH THE SAMPLE IS DRAWN. Biometrika 11: 414–432.

(2) BERTRAND, G.
1906. LE DOSAGE DES SUCRES RÉDUCTEURS. Bull. Soc. Chim. Paris (3) 35: 1285–1299.

(3) BOSWELL, V. R.
1924. CHEMICAL CHANGES DURING THE GROWTH AND RIPENING OF PEA SEEDS. Amer. Soc. Hort. Sci. Proc. 21: 178–187.

(4) ———
1929. FACTORS INFLUENCING YIELD AND QUALITY OF PEAS—BIOPHYSICAL AND BIOCHEMICAL STUDIES. Md. Agr. Expt. Sta. Bull. 306, pp. 341–382.

(5) DANIELS, A. L., and HUTTON, M. K.
1925. MINERAL DEFICIENCIES OF MILK AS SHOWN BY GROWTH AND FERTILITY OF WHITE RATS. Jour. Biol. Chem. 63: 143–156, illus.

(6) FISCHER, E.
1906. UNTERSUCHUNGEN ÜBER AMINOSÄUREN, POLYPEPTIDE UND PROTEÏNE. 1889–1906. v. 1, 770 pp. Berlin.

(7) FRÜHLING, R.
1903. ANLEITUNG ZUR UNTERSUCHUNG DER FÜR DIE ZUCKERINDUSTRIE IN BETRACHT KOMMENDEN ROHMATERIALIEN, PRODUKTE, NEBENPRODUKTE UND HILFSSUBSTANZEN. Aufl. 6, umgearb. und vermehrte, 505 pp., illus. Braunschweig.

(8) FUNK, C.
1908. ÜBER DEN WERT DER ZUR BESTIMMUNG DES HARNZUCKERS VERWENDBAREN METHODEN. Hoppe-Seyler's Ztschr. Physiol. Chem. 56: [507]–511.

(9) GRUBE, K.
1910. QUANTITATIVE ZUCKERBESTIMMUNG MIT HILFE DER KUPFERMETHODEN UND SPEZIELLE METHODEN ZURZ UCKERBESTIMMUNG IN TIERSCHEN FLÜSSIGKEITEN. *In* Abderhalden, E., Handbuch der Biochemischen Arbeitsmethoden, Bd. 2, pp. 167–189, illus. Berlin and Wien.

(10) HERZFELD, A.
1888. DIE ART DER AUSFÜHRUNG DER INVERSIONSMETHODE. Ztschr. Ver. Rübenz. Indus. 38: 699–722.

(11) JODIDI, S. L.
1927. THE NITROGEN COMPOUNDS OF THE RICE KERNEL AS COMPARED WITH THOSE OF OTHER CEREALS. Jour. Agr. Research 34: 309–325.

(12) ———
1931. THE NONPROTEIN NITROGEN OF THE ALASKA PEA, WITH SPECIAL REFERENCE TO THE CHEMICAL NATURE OF HUMIN NITROGEN. Jour. Agr. Research 43: 811–825.

(13) ——— KELLOGG, E. H., and TRUE, R. H.
1918. NITROGEN METABOLISM IN NORMAL AND IN BLIGHTED SPINACH. Jour. Agr. Research 15: 385–408.

(14) ——— MOULTON, S. C., and MARKLEY, K. S.
1920. THE MOSAIC DISEASE OF SPINACH AS CHARACTERIZED BY ITS NITROGEN CONSTITUENTS. Jour. Amer. Chem. Soc. 42: 1061–1070.

(15) ——— MOULTON, S. C., and MARKLEY, K. S.
1920. A MOSAIC DISEASE OF CABBAGE AS REVEALED BY ITS NITROGEN CONSTITUENTS. Jour. Amer. Chem. Soc. 42: 1883–1892.

(16) ——— and PEKLO, J.
1929. SYMBIOTIC FUNGI OF CEREAL SEEDS AND THEIR RELATION TO CEREAL PROTEINS. Jour. Agr. Research 38: 69–91.

(17) LOHRISCH, H.
1911. METHODEN ZUR UNTERSUCHUNG DER MENSCHLICHEN FÄZES. *In* Abderhalden, E., Handbuch der Biochemischen Arbeitsmethoden Bd. 5, Teil 1, pp. [331]–420, illus. Berlin and Wien.

(18) OSBORNE, T. B., and CAMPBELL, G. F.
1896. LEGUMIN AND OTHER PROTEIDS OF THE PEA AND THE VETCH. Jour. Amer. Chem. Soc. 18: [583]–609.

(19) ——— and CAMPBELL, G. F.
1898. PROTEIDS OF THE PEA. Jour. Amer. Chem. Soc. 20: 348–362.

(20) ——— and CAMPBELL, G. F.
1898. THE PROTEIDS OF THE PEA, LENTIL, HORSE BEAN, AND VETCH. Jour. Amer. Chem. Soc. 20: 410–419.

(21) ROSENBLATT, M.
1912. ÜBER DIE QUANTITATIVE BESTIMMUNG VON GLUCOSE BEI GEGENWART VON FREMDEN STOFFEN NACH DER ANALYTISCHEN METHODE VON GABRIEL BERTRAND. Biochem. Ztschr. 43: [478]–480.

(22) ———
1913. BEMERKUNG ZU EINER MITTEILUNG VON G. SONNTAG: "DIE METHODE VON GABRIEL BERTRAND ZUR ZUCKERBESTIMMUNG". Biochem. Ztschr. 57: [335]–336.

(23) SAYRE, C. B., WILLAMAN, J. J., and KERTESZ, Z. I.
1931. FACTORS AFFECTING THE QUALITY OF COMMERCIAL CANNING PEAS. N.Y. Agr. Expt. Sta. Tech. Bull. 176, 76 pp., illus.

(24) SCHMIDT, E.
1901. AUSFÜHRLICHES LEHRBUCH DER PHARMAZEUTISCHEN CHEMIE. Aufl. 4, Bd. 2, Abt. 1, 1020 pp., illus. Braunschweig.

(25) SCHULZE, E., STEIGER, E., and MAXWELL, W.
1891. UNTERSUCHUNGEN ÜBER DIE CHEMISCHE ZUSAMMENSETZUNG EINIGER LEGUMINOSENSAMEN. Landw. Vers. Sta. 39: [269]–326.

(26) STUTZER, A.
1880. UNTERSUCHUNGEN ÜBER DIE QUANTITATIVE BESTIMMUNG DES PROTEIN-STICKSTOFFS UND DIE TRENNUNG DER PROTEINSTOFFE VON ANDEREN IN PFLANZEN VORKOMMENDEN STICKSTOFFVERBINDUNGEN. Jour. Landw. 28: 103–123.

(27) ZEMPLÉN, G.
1912. DARSTELLUNG, GEWINNUNG, NACHWEIS UND BESTIMMUNG DER HÖHEREN KOHLENHYDRATE. *In* Abderhalden, E., Handbuch der Biochemischen Arbeitsmethoden, Bd. 6, pp. 1–82, illus. Berlin and Wien.

ABNORMALITIES IN THE FLOWER AND FRUIT OF CAPSICUM FRUTESCENS [1]

By H. L. COCHRAN [2]

Assistant in Department of Vegetable Crops, New York (Cornell) Agricultural Experiment Station

INTRODUCTION

Relatively few cases of abnormalities in *Capsicum frutescens* have received attention in the literature. The cases reported are scattered over a period of more than half a century. No detailed historical study has apparently been made to differentiate between abnormalities infrequently occurring in the flower and the normal flower itself, and only in the paper by Harris (*6*)[3] has any such work been reported on those more frequently found in the fruit. It is the object of this paper, therefore, to review the literature confined to abnormalities in the genus *Capsicum*, and to present a more detailed study of those occurring in both the flower and the fruit.

The work reported here was done in connection with some studies that are being carried out on fruit setting in the pepper (*Capsicum frutescens* L.) as influenced by certain environmental factors.

LITERATURE REVIEW

The earliest and yet perhaps the most extensive investigation concerning abnormalities in the genus *Capsicum* is that of Terracciano (*15*), who noted the changing of stamens into carpels and a growing together of these with the pistil in the flower of *C. grossum*. He also reported two types of abnormalities in the fruit of *C. annuum*. In one form the walls of the fruit, terminated by a hollow tubiform style open at the top, showed five protuberant evaginations. Internally the fruit produced no seeds but contained five similar fruits, likewise without seeds and with short styles having almost perfect stigmas, disposed upon the apex of the thalamus.

Mottareale (*10*) reported adesmy of the carpels in several cases, and stamens that were both larger and smaller than the corolla pieces. He also saw diaphysis and ecblastesis of the fruit. Both abnormalities were in a few cases seen in the same fruit. Mottareale cites Terracciano as having established that the staminate vasal collar develops externally like the corolla and is capable in *Capsicum grossum* of generating carpels. Not only are the carpels formed at the alternating petalous points, transformation of the normal stamens, but also at the epipetalous points, forming small fruits by development and also by a change of the basal teeth with which every stamen is provided.

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[3] Reference is made by number (italic) to Literature Cited, p. 747.

Heckel (*8*) observed pistillody of the stamens and a coalescence of them with the pistil in flowers of *Capsicum annuum*. He also reported that abnormalities in the fruit were relatively common. He found only a single carpel in some, while in others there were present more or less well formed fruits.

Some unusual types of abnormalities have been reported, chief among which is one by Schilberszky (*13*). From a common pedicel two individual fruits of *Capsicum annuum* arose and were grown together at the lower side in an angle of about 80°. Both fruits were equal in size and normally developed. The calyx which usually has 5 points, in this case had 10 and was forced between the single fruits in such a manner that it formed an obtuse angle.

Gallardo (*2*) has described a case in *Capsicum annuum* where internal tuberlike formations as large as 2.5 cm in diameter appeared, which he thought resulted from seeds becoming swollen. Welter (*17*) found that very often the seeds of *C. annuum* sprouted in the fruit.

Viviand-Morel (*16*) reported a small internal fruit in the pepper which originated from the apex of the axis, while Halstead (*4, 5*) noted an almost identical case of a miniature included fruit that arose from the apex of the fleshy columella on which the seeds are borne. It resembled a normal fruit very much and was green. Raymondaud (*12*) also noted a small, globular, olive-shaped internal fruit in *Capsicum annuum*.

Penzig (*11, v. 2, p. 174*) reported, after a long study, that abnormalities occurred rather frequently in the fruit of the genus *Capsicum*. Penzig (*11, v. 3, p. 77*) cites Borbás (*1*) as having found central prolification of the fruit in the same genus. This is also borne out by the findings of Harris (*6*). Irish (*9*) in his extensive work with the pepper has reported similar abnormalities in the fruit.

Sturtevant (*14*) reported that sweet peppers were subject to the development of a berry or berries within the berry. These enclosed fruitlike bodies were in some cases found to produce a few seeds.

MATERIALS AND METHODS

During 1932–33 two crops of peppers were grown in the experimental greenhouses of the Department of Vegetable Crops at Cornell University. Plants from which material was collected were of the World Beater variety grown both in bank sand, to give a low-nitrogen series, and in soil high in organic matter to which sodium nitrate was added at weekly intervals to give a high-nitrogen series, in 1-gallon glazed crocks. One plant was placed in each crock and grown under two controlled temperature conditions, medium 60°–70° F., and warm 70°–80°. Fresh material was observed under a wide-field binocular microscope. Slides were made of paraffin sections, the material being killed and fixed in a solution made as follows: Solution A, 1 g chromic acid and 10 cc glacial acetic acid in 90 cc of water; solution B, 40 cc of commercial 40 percent formalin added to 10 cc of water (Karpechenko's chromo-acetic). Equal parts of the two solutions were mixed at the time they were used. Heidenhain's iron-alum haematoxylin was found very satisfactory for staining all tissues studied in the histological part of the investigation.

TERATOLOGICAL TRANSITIONS IN THE FLOWER

The normal flower of *Capsicum frutescens* (fig. 1, *D*) occurs either singly or 2 or 3 together in the axils of branches. It has a minutely 5-lobed calyx and a rotate corolla that is also 5-merous. The syle is linear, straight, longer than the stamens, and terminated by a subcapitate stigma. There are 5 stamens attached to the ovary

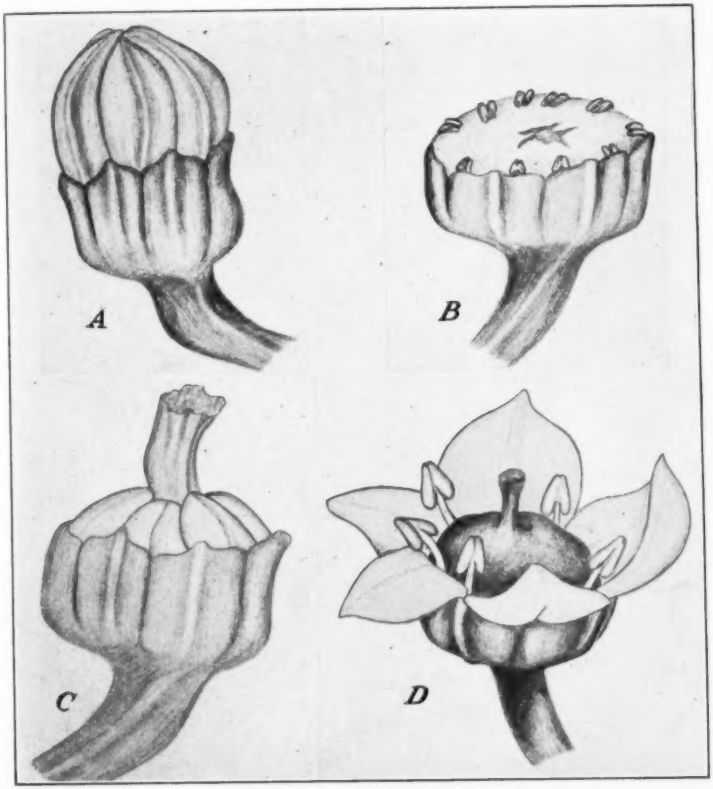

FIGURE 1.—*A*, Normal bud 1 day prior to anthesis; *B*, abnormal bud devoid of pistil (note contorted area where pistil usually is attached) and stamens devoid of filaments; *C*, young abnormal bud with protruding style, to be compared with *A*; *D*, normal flower on date of anthesis.

near the base of the corolla having heart-shaped anthers that dehisce by longitudinal splitting. The ovary is usually 3-celled.

In the case of the abnormal flower (fig. 2, *A*) which is ordinarily easily recognized, some interesting teratological changes sometimes take place. The calyx may undergo partial metamorphosis, thus exhibiting almost unconceivable transitions. One very abnormal case was noted in this study when a calyx lobe enlarged and assumed the appearance of an almost fully developed petal (petalody), while another calyx lobe on the same flower developed into a short pistil (pistillody). According to Gray (*3*, *p. 174*) pistillody from parts

other than the stamens rarely occurs. Some abnormal flowers have normal calyxes, i.e., 5-lobed, others have as many as 8, while still others, as was noted by Mottareale (*10*) and the writer, may be completely deprived of the calyx.

A great variation was found in the stage of development of the flower at which the transformations occur as well as the time taken

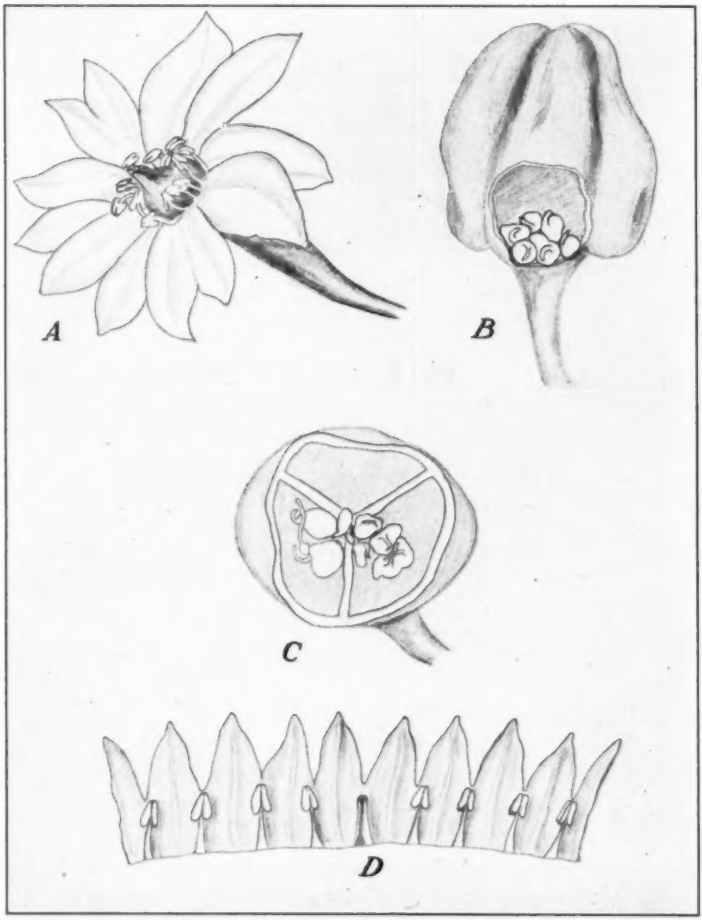

FIGURE 2.—*A*, Abnormal flower with many petals, some divided, and 13 stamens; *B–C*, mature fruits showing internal fruitlike bodies on receptacle; *D*, abnormal corolla with 9 stamens attached, of which 8 are normal, and 1 lacks the anther, but has instead a round hole extending to bottom of filament.

for their completion. Staminody may be initiated in the young bud stage and completed during that time, or it may not start until about the time of anthesis. The abnormal phenomenon may cease at this stage, leaving a contorted-appearing organ or after a time complete itself.

As is shown in figure 3 the abnormal corolla does not shed within 2 or 3 days or become withered, like that of the normal flower. It persists until the ovary develops to the point that the basal collar is split, thus allowing the corolla to drop.

While the majority of abnormal flowers contain from 7 to 9 stamens, occasionally there may be more. In figure 2, *A* is shown a flower with 13 normal stamens, whereas in figure 1, *B*, is shown another flower with 10 anthers completely deprived of their filaments. In figure 2, *D*, is a flower with 9 stamens, 8 normal ones and another devoid of its anther but with an opening at the apex that extends to the bottom of the filament. A similar case of the latter abnormality was reported by Mottareale (*10*).

Stamens sometimes undergo partial retrogressive metamorphosis thus causing some of them to unite with the petals, and they may even

FIGURE 3.—Plant of *Capsicum frutescens* showing abnormal corollas which persist in being attached.

take the appearance of petals but may not complete the transition to petalody. However, this completion was observed during the course of this study.

The abnormal style is perhaps the most easily recognized flower part. Normally it can be detected in the early bud stage by its unusual protrusion above the tightly closed petals. It is generally short and broad, and often severely contorted as is shown in figure 1, *C*. Complete anthesis of such flowers is delayed, and owing to the early emergence of the stigmatic surface beyond the stamens, the chances for self-pollination are very small. Owing to this and to the fact that sometimes such stigmatic surfaces fail to become receptive, the ovules are not fertilized and the fruits develop parthenocarpically.

However, cross-pollination and perhaps self-pollination, evidently take place in a few cases. A point of great interest at this stage is the behavior of such styles with reference to their development and abscission. Normal styles at the time of anthesis are rarely more than 1 mm in diameter near the base and 5 mm in length. The styles usually dry up and drop soon after fertilization has taken place. Very often the style of an abnormal flower has become 2 to 2.5 mm in diameter and 7 to 9 mm in length by the time the flower opens. It does not abscise but invariably remains attached to the ovary, develops chlorophyll, and increases in size with the ovary until maturity, at which time it takes on the normal red color. The development of the various stages of the abnormal style is shown in figure 4. Occasionally flowers develop devoid of styles as can be seen in figure 1, *B*. There is usually a more or less contorted cavity at the apex of the ovary in such fruits. The ovary may in some cases split longitudinally into a rosette of stylar portions, some separate, other united. Each portion may develop a stigmatic surface which

FIGURE 4.—Development of the abnormal style; it does not abscise but continues in growth with the ovary. Corollas have been removed from the larger fruits.

appears to be very abnormal. Such conditions are not found very frequently, however, and the stage to which they develop depends on the age of the ovary.

TERATOLOGICAL TRANSFORMATIONS IN THE FRUIT

Harris (7) in working with okra, states:

> Among the various phenomena that may be included by the teratologists under the term "prolification of the fruit", one of the most interesting is the production of a more or less completely formed second fruit inside the first. Generally, the included "fruit" is distinctly abnormal in character, often reduced to a whorl, or a series of whorls, of irregularly formed and usually sterile carpels.

The fruitlike structures in *Capsicum frutescens* apparently have no definite form but vary from an irregular contorted body through an almost perfectly formed sterile fruit, comparable in shape with the one in which it is produced, to linear bodies from a few millimeters to 2 cm in length, some of which are terminated by a minute style. A few of the fruitlike bodies noted, however, contained no styles, as is shown in table 1. It is also apparent from these data that other bodies contained more than one style. Several bodies had as many as three styles, and they were not always normal in form as some were very much fasciated. In one case there were four styles. One style, however, was practically always found attached at the apex of the body while the others were usually formed near the apex or on the side. This is shown in figure 2, *B*, *C*.

TABLE 1.—*Distribution of abnormalities in the fruits of Capsicum frutescens*

Lot no.	Date fruits were harvested	Temperature under which plants were grown	Fruits harvested	Normal fruits	Abnormal fruits	Fruits abnormal	Internal abnormalities	Abnormalities having styles	Styles contained	Seeds in abnormal fruits
		° *F.*	*Number*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*
1	Dec. 7, 1932	60-70	191	188	3	1.57	71	50	76	306
2	Dec. 26, 1932	70-80	129	123	6	4.65	55	42	78	14
3	Jan. 11, 1933	70-80	213	204	9	4.22	69	43	54	34
4	Jan. 21, 1933	60-70	175	170	5	2.85	33	25	61	12
5	Feb. 4, 1933	70-80	163	156	7	4.29	37	28	54	32
6	Feb. 16, 1933	60-70	228	217	11	4.82	34	16	32	28
7	Feb. 28, 1933	60-70	195	187	8	4.10	22	15	21	95
8	Mar. 19, 1933	70-80	142	132	10	7.04	36	19	32	1
9	May 6, 1933	70-80	223	216	7	3.13	15	10	21	280
10	May 19, 1933	(a)	216	202	14	6.48	50	40	86	9
11	July 13, 1933	(a)	147	125	22	14.96	55	41	81	75
Total			2,022	1,920	102	5.04	477	329	596	886

a Temperature uncontrolled.

The data in table 1 show a seasonal distribution of the abnormalities in the fruits. Even though the percentage of abnormal fruits was not unusually high at any one time, abnormal fruits were present throughout the duration of the experiment. The number of internal bodies was in no way constant. In fact, the determination of such was sometimes rather difficult because of the varying degrees of fusion of the members of the outer whorls and the small size of those of the inner ones. Harris (*6*) counted the number of styles and used this as a criterion of the number of internal abonormalities in *Passiflora*, but the writer found the method unsatisfactory in the present study because some of the included bodies contained more than one style. In the majority of cases reported here, counts were made under a wide-field binocular microscope using a dissecting needle to separate the sometimes much entangled fruitlike structures. Some fruits contained only 1 of these structures, while others had as many as 18 to 20, and in one case 22 of such abnormalities were noted. They completely filled the fruit cavity.

HISTOLOGICAL DIFFERENTIATIONS BETWEEN PARTS OF THE NORMAL FLOWER AND FRUIT AND EXISTING ABNORMALITIES

Since no previous work has been reported that differentiates histologically between abnormalities infrequently occurring in the flower and the normal flower itself, attention has naturally been focused on this relationship here. Figure 5, *A* and *B*, shows cross sections of a normal style and the style of an internal fruitlike body. Both are similar in some tissues, yet as a whole their development and tissue arrangement resemble each other but very little. The style of the abnormal fruit failed to develop a stylar canal. This fact may offer a partial explanation for the failure of the internal abnormal fruitlike bodies to become fertilized, and to form seed even when pollen has been applied to the stigmalike surface. Some abnormal styles were found to contain only 1 vascular bundle while others were frequently noted that contained from 6 to 8. The usual number of vascular bundles found in the normal style is 4.

The conducting tissue through which the pollen tubes pass is usually found immediately surrounding the stylar canal as is shown in figure 5, *A*. In the abnormal style it may be completely absent or found to

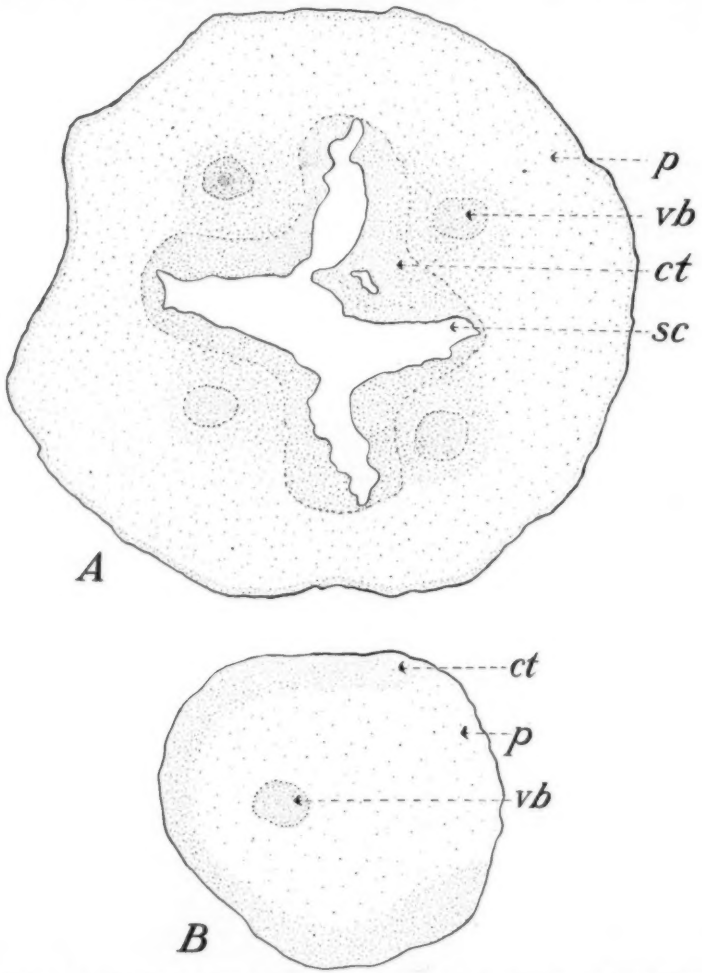

FIGURE 5.—*A*, Cross section of normal style: *p*, Parenchyma; *vb*, vascular bundle; *ct*, conducting tissue; *sc*, stylar canal. *B*, Cross section of abnormal style of internal fruitlike body. ×178.

one side and sometimes mixed in with the parenchyma tissue. This can be seen in figure 5, *B*.

Harris, (*6*, *p. 137*) in his mention of the histological similarity existing between carpellike bodies and the wall of the fruit, writes:

Both show the exceedingly heavy epidermis and the large-celled parenchymatous ground tissue. The bodies show one or more vascular bundles similar to

those of the wall of the fruit. When fresh sections are examined, numerous chloroplasts are seen in both but they are generally more abundant in the wall of the fruit.

That the wall of the internal fruitlike body is similar histologically to the wall of a normal fruit is shown in figure 6, *A* and *B*. There are

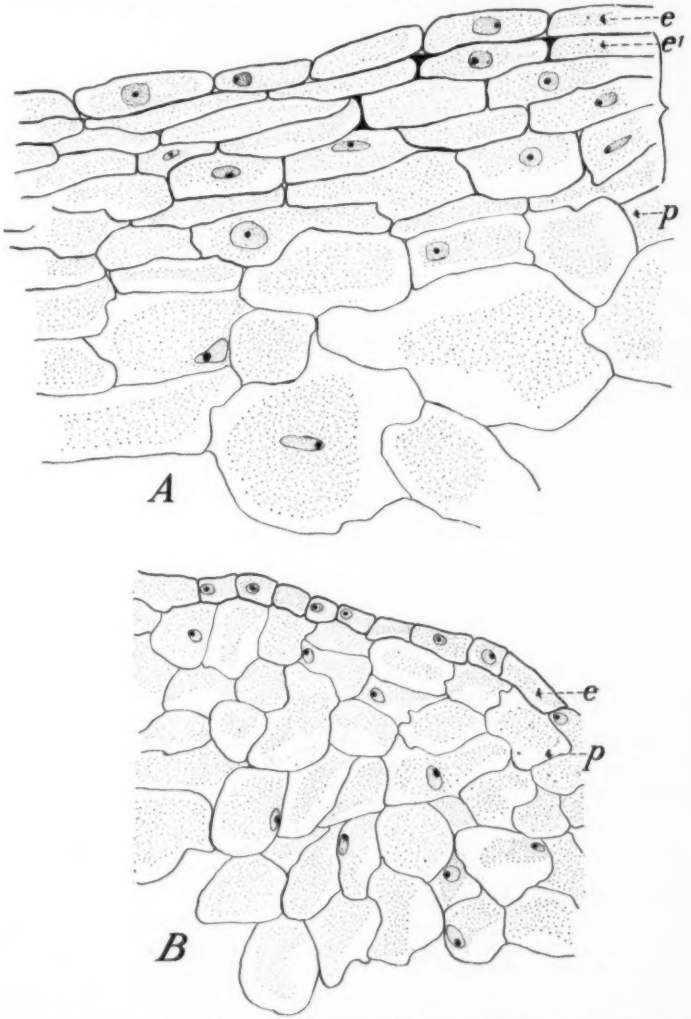

FIGURE 6.—*A*, Cross section of wall of normal fruit: *e*, Epidermis; *e'*, epicarp; *p*, parenchyma. *B*, Cross section of wall of internal fruitlike body. × 750.

some differences noted, however, in that the wall of the fruit contains several layers of cells constituting the epicarp which are absent in the wall of the fruitlike body. The former also contains much larger

parenchyma cells than does the latter. The chloroplasts and vascular bundles were similar in both cases.

DISCUSSION

The question as to what factors cause the development of abnormalities has probably arisen in the minds of all workers who have reported them, but up to the present time explanations are lacking. Mottareale (*10*) is the only previous worker who has attempted to correlate environmental conditions with the existence of these abnormalities. He attributed the abnormalities found in the flower and fruit of *Capsicum annum* and *C. grossum* to short periods of cold weather under which the plants were grown. This explanation, however, does not seem altogether feasible, since he later repeated the same treatment twice and was unable to obtain similar results. Work of the writer tends to disprove the idea that low temperature is the only factor that results in the initiation and production of abnormalities. Those that are reported here were produced on plants grown under controlled medium (60° to 70° F.) and high (70° to 80°) temperatures in greenhouses during the winter of 1932, and under uncontrolled temperatures (70° to 110°) in greenhouses during the summer of 1933. This does not prove, however, that abnormalities would not be initiated under still lower temperatures than those used in this study. The data in table 1 show that the number as well as the percentage of abnormal fruits was greater during the summer under high uncontrolled temperatures than at any other time of the year. This fact suggests that high temperature is more effective in producing abnormal fruits than is medium temperature. In addition, the results of examination of a large number of flowers during the same periods agree with these findings in regard to the fruits.

From the beginning of this study indications seemed to show that a competition for nutrients or elaborated food between the rapidly growing normal plant parts may be the cause of the initiation of abnormalities since they appeared first on plants in the low-nitrogen series. Soon afterwards, however, several abnormal fruits and one abnormal flower were noted on plants in the high-nitrogen series. In fact, by the time the experiment terminated plants in the high-nitrogen series had produced more abnormalities than those in the low-nitrogen series, probably because the former produced more flowers and fruits.

SUMMARY

A review of the literature concerning abnormalities in the genus *Capsicum* and a detailed study of those occurring in both the flower and the fruit are reported here.

A very rare case was noted in which two calyx lobes on the same flower assumed different teratological transformations, namely, pistillody and petalody.

There is no definite stage in the formation of the flower at which the transitions are initiated or completed.

The abnormal corolla does not drop or wither within 2 or 3 days but persists until the ovary develops to the point that the basal collar splits and this allows the corolla to drop.

Stamens may undergo partial retrogressive metamorphosis, thus causing some of them to unite with the petals, and may even take

the appearance of such, but may not complete the transition to petalody.

The abnormal style can commonly be detected in the early bud stage by its unusual protrusion above the tightly closed petals. It does not absciss but remains attached to the ovary, develops chlorophyll, and increases in size with the ovary until maturity, at which time it takes on the normal red color.

The internal fruitlike bodies are usually distinctly abnormal in character. Some have no style, while other have as many as 3, and in one case 4 were noted.

The number of internal abnormalities was in no way constant, as they ranged all the way from 1 to 22.

Histological differentiations are made between parts of the normal flower and fruit and existing abnormalities.

Both the style of a normal flower and that of an interal fruitlike body are similar in some tissues, yet as a whole their development and tissue arrangement parallel each other but very little. The fruitlike bodies failed to develop any indications of a stylar canal.

Histological similarities between the wall of the internal fruitlike body and the wall of the fruit are interesting. Both have a heavy epidermis, large parenchyma cells, chloroplasts, and vascular bundles. There is one very distinct difference, however, in that the fruit wall contains several layers of cells, which make up the epicarp and which are absent in the wall of the carpel like body.

While both abnormal flowers and fruits were produced more abundantly on plants growing under high and high uncontrolled temperatures, they appeared to some extent on plants under medium temperature.

LITERATURE CITED

(1) Borbás, V.
1880. fias paprikagyü möles. Földmüvelèsi Érdekeink 45: 459.

(2) Gallardo, A.
1902. observaciones morfológicas y estadísticas sobre algunas anomalias de digitalis purpurea l. An. Mus. Nac. Hist. Nat. Buenos Aires 7: [37]–72, illus.

(3) Gray, A.
1879. structural botany, or, organography on the basis of morphology, to which is added the principles of taxonomy and phytography, and a glossary of botanical terms. Ed. 6, pt. 1, 442 pp., illus. New York and Chicago.

(4) Halstead, B. D.
1891. a strange thing on peppers. Bull. Torrey Bot. Club 18: 151, illus.

(5) ———
1893. some vegetable malformations. Pop. Sci. Monthly 42: 318–325, illus.

(6) Harris, J. A.
1906. prolification of the fruit in capsicum and passiflora. Mo. Bot. Gard. Ann. Rept. 17: 133–145, illus.

(7) ———
1913. prolification of the fruit in okra, hibiscus esculentus. Torreya 13: 33–35.

(8) Heckel, E.
1882. nouvelles monstruosités végétales. Bull. Soc. Bot. France (2) 29: 292–312, illus.

(9) Irish, H. C.
1902. variations in some introduced garden vegetables. Soc. Prom. Agr. Sci. Proc. 23: [63]–64.

(10) MOTTAREALE, G.
1904. GELATE E FENOMENI CLEISTOGAMICI E TERATOLOGICI NEL SOLANUM MELONGENA E NEL CAPSICUM ANNUUM E C. GROSSUM. Ann. R. Scuola Agr. Portici (2) 6: 1–22, illus.

(11) PENZIG, O.
1894–1922. PFLANZEN TERATOLOGIE, SYSTEMATISCH GEORDNET. 2 v. (1894 ed.); Aufl. 2, stark verm., 3 v. (1921–22). Berlin.

(12) RAYMONDAUD, E.
1904. ENDOCARPIE (INCLUSION D'UN PIMENT DONS UN PIMENT). Rev. Sci. Limousin 12: 369–372.

(13) SCHILBERSZKY, K.
1885. CORRESPONDENZ. Oesterr. Bot. Ztschr. 35: 408–409.

(14) STURTEVANT, L. E.
1890. SEEDLESS FRUITS. Mem. Torrey Bot. Club 1: [14]–185.

(15) TERRACCIANO, N.
1878. INTORNO ALLA TRASFORMAZIONE DEGLI STAMI IN CARPELLI ENL CAPSICUM GROSSUM, E DI UN CASO PROLIFICAZIONE FRUTTIPARA NEL CAPSICUM ANNUUM. Nuovo Gior. Bot. Ital. 10: 28–33, illus.

(16) VIVIAND-MOREL, J. V.
1902. GROS PIMENT CARRÉ, DOUX, PROLIFÈRE. Lyon Hort. 24: 382–383, 385, illus.

(17) WELTER, H.
1881. [UNE MONSTRUOSITÉ DES FRUITS DE CAPSICUM ANNUUM.] Bull. Trav. Soc. Bot. Genève (1879–80) 1881: 39–40.

SOIL TREATMENT IN RELATION TO CLUBROOT OF CABBAGE [1]

By R. H. LARSON, *assistant in plant pathology, University of Wisconsin*, and formerly *agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry*, and J. C. WALKER, *professor of plant pathology, University of Wisconsin, and agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry*

INTRODUCTION

The investigation herein described is a continuation of work carried on by Wellman [2] during the years 1924 to 1928 inclusive. In a report of his studies he noted that the hydrate was the most effective compound of calcium used to control clubroot of cabbage. In 1928 he secured good control of the disease on severely infested soil in Kenosha County, Wis., with a 2-ton treatment of this chemical. In 1930 and 1931 field treatments were carried out by the writers on infested soil in the same locality. Since in these cases even heavier treatments of the soil with calcium hydrate had little effect upon clubroot infection, further studies were undertaken to determine, if possible, what factors might influence the effectiveness of liming materials.

Previous work on the relation of soil reaction to infection by the clubroot organism (*Plasmodiophora brassicae* Wor.) has been reviewed by Wellman. Chupp [3] and Wellman [4] both succeeded in completely inhibiting infection in the greenhouse by applying calcium hydrate in sufficient quantities to make the soil reaction slightly alkaline. In the field experiments results were not always consistent. Ravn [5] secured considerable reduction in infection by the use of finely divided $CaCO_3$ and still better control with a mixture of $CaCO_3$ and CaO. It is important to note, however, that in some cases severe infection occurred and he was unable to explain the variation. Chupp [6] failed to secure effective control in the field with calcium hydrate in the early part of the season, although late-season plantings on the same soil were much less severely infected. Wellman secured complete inhibition of infection in the greenhouse with a 2-ton application of calcium hydrate, but this treatment did not preclude root infection in the field, although it did give very good control. In an examination of the numerous reports of liming experiments with clubroot, one is impressed most by the lack of consistent success in disease control in the field.

[1] Received for publication Dec. 8, 1933; issued June, 1934. Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture.

[2] WELLMAN, F. L. CLUBROOT OF CRUCIFERS. U.S. Dept. Agr. Tech. Bull. 181, 32 pp., illus. 1930.

[3] CHUPP, C. CLUBROOT IN RELATION TO SOIL ALKALINITY. Phytopathology 18: 301-306, illus. 1928.

[4] WELLMAN, F. L. See footnote 2.

[5] RAVN, F. K. FORSØG MED ANVENDELSE AF KALK OG KUNSTRGØDNING SOM MIDDEL MOD KAALBROKSVAMP. Tidsskr. Landbr. Planteavl 17: [163]-177. 1910.

——— FORSØG MED ANVENDELSE AF KALK SOM MIDDEL MOD KAALBROKSVAMP. Tidsskr. B and br. Planteavl 18: [357]-392. 1911.

[6] CHUPP, C. See footnote 3.

FIELD EXPERIMENTS

The field experiments discussed herein were conducted on two badly infested fields. One of these was located near Somers, Kenosha County, Wis., and will be referred to hereafter as the Miller plot. The other was located near Franksville, Wis., and is designated as the Franksville plot. The Miller plot was nearly level except for a low swale which passed through it. The soil was a well-drained Carrington silty clay loam, quite uniform in consistency. The Franksville plot had a slight irregular slope, and the soil was a Clyde silty clay loam. The commercial cabbage crop of 1930 on this area was quite generally infected, but the infection was noticeably least severe on the lower portion of the slope.

EXPERIMENTS ON THE MILLER PLOT

1930 Trials

A heavy application of manure was made before plowing. After the land was prepared, 3–10–10 fertilizer was disked in thoroughly at the rate of 1,000 pounds per acre. The liming materials were applied and disked into the soil on June 9 and cabbage (*Brassica oleracea capitata* L.) plants were set on June 20. Two types of hydrate were used. One, which will be referred to as calcium hydrate, had been prepared from limerock consisting of about 95 percent calcium carbonate. The other, dolomitic hydrate, was prepared from dolomitic limestone which contained approximately equal amounts of calcium carbonate and magnesium carbonate.

At the time of liming, the soil was relatively dry and it remained so until transplanting. For 2 weeks after transplanting there was no rain, and the only addition of water during this period was that applied around the plants when they were set. Thus the conditions following transplanting were, according to Monteith,[7] those least favorable for infection, although the "watering-in" of the plants would, according to Wellman[8], be sufficient to favor a certain amount of infection.

The reaction of the soil before lime was applied was pH 5.5, and at the end of the season in the untreated area it was pH 5.8. In the 2-ton calcium hydrate treatment the reaction of the soil changed from pH 5.5 to 7.1, and in the 4-ton treatment to 7.4. In the case of dolomitic hydrate the pH of the soil was somewhat higher, shifting from 5.5 to 7.2 in the 2-ton treatment and to 7.6 in the 4-ton treatment.

General infection became evident by the flagging of the plants during the middle of the day about 8 weeks after they were transplanted. This distinguishing symptom of clubroot infection was apparent over most of the plot. At this time there appeared to be little difference between the treated and untreated areas. It is worthy of note, however, that the cabbage on the area treated with dolomitic hydrate, 4 tons per acre, produced somewhat larger plants than did those on the areas receiving the other treatments.

After the yield data were taken, a large number of plants in each of the treated and untreated areas were pulled and the roots examined to

[7] Monteith, J., Jr. Relation of soil temperature and soil moisture to infection by plasmodiophora brassicae. Jour. Agr. Research 28: 549–562, illus. 1924.
[8] Wellman, F. L. See footnote 2.

determine the amount and severity of the disease. All the roots examined were more or less clubbed. There was no measurable difference in the extent of infection in the treated and the untreated areas except that in the former the clubs were usually intact, whereas in the latter they were usually completely decayed, indicating that infection has taken place earlier.

Less than 2 percent of the plants in the treated areas produced marketable heads. The amount of practical control was negligible, and no measurable differences in yield between the four treated areas and the untreated area were secured except on the low area in one portion of the field. In this area, which was favored with a higher level of soil moisture during the growing season, some evidence of the action of the lime as a disease inhibitor was observed. The plants made much better growth as the season progressed, and when the root systems were examined at the end of the growing period a large percentage of plants from the limed portion showed complete inhibition of infection, while in the unlimed portion infection was general.

It is evident from the results of this season that even though calcium and dolomitic hydrate were applied in amounts sufficient to change the reaction of the soil to well above pH 7.0, the amount of infection in most of the plot was not materially affected. This suggested that seasonal or soil conditions influenced the effectiveness of the hydrate in some way not indicated by the pH reading.

1931 Trials

In 1931 portions of the same treated and untreated areas of the Miller plot were replanted without further application of liming materials. Another portion of the same field was laid out for further trials. To this various amounts of calcium hydrate and Agstone (finely ground dolomitic limestone) were applied in the autumn of 1930. The field was planted to cabbage on June 1, 1931. Rainfall was again below normal during the growing season.

Table 1.—*Effect of various soil treatments upon soil reaction and yield of cabbage on a clubroot-infested soil; Miller plot, 1931*

Material applied	Rate of application per acre	Date of application, 1930	Soil reaction			Yield, September 1931		
			August 1930	June 1931	September 1931	Plants	Marketable heads	Weight of heads per acre
	Tons		*pH*	*pH*	*pH*	*Number*	*Percent*	*Tons*
None	0		5.5	5.8	5.8	2,110	1	0.34
Dolomitic hydrate	2	June 9	7.2	7.1	7.0	246	6	.25
	4	do	7.6	7.4	7.4	243	11	.34
	2	do	7.0	7.0	6.8	249	4	.12
	4	do	7.4	7.2	7.2	240	5	.15
Calcium hydrate	1	Oct. 22	5.5	7.2	7.0	961	17	1.42
	2	do	5.5	7.3	7.1	968	23	2.08
	1	do	5.5	7.1	7.1	971	28	1.98
	2	do	5.5	7.4	7.2	965	26	2.98
Agstone	4	do	5.5	7.4	7.2	462	3	.17
	8	do	5.5	7.8	7.6	471	4	.24

In table 1 is shown the reaction of the soil in the various plots in August 1930, at the time of transplanting in June 1931 and at the end of the season in September 1931. At the time the plants were set the

soil in all treated plots was neutral or above. In fact there was then little difference in soil reaction between the plots that had been treated with Agstone and those that had been treated with hydrate in October 1930, or between the plots treated with hydrate in June 1930 and those treated with hydrate in October 1930. In spite of this there was no appreciable reduction in clubroot infection in the hydrate-treated plots of June 1930, nor in the Agstone-treated plots of October 1930. While infection was general in the hydrate plots of October 1930, there were distinct benefits from these treatments, as is shown by the percentage of marketable heads produced. Even in these cases, however, a commercially profitable degree of control was not attained.

EXPERIMENTS ON THE FRANKSVILLE PLOT

1931 Trials

The Franksville plot was plowed in the autumn of 1930, and to certain portions of it 1½ and 2½ tons of calcium hydrate per acre and 4 and 8 tons of Agstone per acre were applied on October 22, 1930. Further treatments of calcium hydrate were applied to other portions at the rate of 1½ and 2½ tons per acre the following spring, one series of treatments being made on April 25, as early as seasonal conditions permitted, and the other on June 2, shortly before the entire plot was planted on June 5.

TABLE 2.—*Effect of various soil treatments upon soil reaction and yield of cabbage on a clubroot-infested soil; Franksville plot, 1931*

Material applied	Rate of application per acre	Date of application	Soil reaction		Yield, September 1931	
			September 1930	September 1931	Plants	Marketable heads
	Tons		*pH*	*pH*	*Number*	*Percent*
None	0		6.2	6.3	2,078	2
Agstone	4	Oct. 22, 1930	6.2	7.0	864	3
	8	do	6.2	7.4	870	5
Calcium hydrate	1½	do	6.2	7.0	2,074	25
	2½	do	6.2	7.2	2,170	34
	1½	Apr. 25, 1931	6.2	7.0	571	27
	2½	do	6.2	7.2	507	37
	1½	June 2, 1931	6.2	7.1	574	30
	2½	do	6.2	7.2	572	41

In table 2 the effect of the various treatments upon soil reaction and upon yield of marketable heads is given. All treatments changed the reaction to neutral or above. The heavier Agstone treatment gave the highest pH reading, but there was little effect upon clubroot as measured by the percentage of plants which produced marketable heads. The calcium hydrate treatments as a whole were much more effective, the heavier of these treatments raising the pH slightly and giving somewhat higher percentages of marketable heads. None of the hydrate treatments gave what might be considered a commercially successful control. Although there was some reduction in the amount of clubbing as compared with that on the untreated area and on the areas treated with Agstone, the roots of most plants were more or less infected.

The results obtained in 1931 on the Miller plot suggested that fall application might be more effective since in this case it was more influential than the treatment of the spring of 1930. Chupp[9] attributed increasing effectiveness of hydrate during the current season to the time required for the hydrate to become active. In the Franksville experiment a direct comparison between fall, early spring, and late spring applications of hydrate is made. No indication is given that a long interval between application and planting enhanced the inhibition of infection. In fact, the last application, made 3 days before planting, gave slightly better results, although the actual differences are negligible.

1932 Trials

The Franksville plot was replanted in 1932, with no further additions of lime. The purpose was to determine whether or not the treatments of 1931 would affect clubroot infection the second season after application. The rainfall was somewhat heavier and more evenly distributed than in 1931. As the season progressed it became increasingly evident that the disease was less severe in the lower portions of the field regardless of treatment. In the final collection of data the percentage of infection and marketable heads was estimated in high and low portions of the same treated area where such an area traversed both levels. The data thus secured are given in table 3. In general the amount of infection was slight in the low portions of the plot and severe in the high portions, irrespective of treatment, and there was no direct correlation between soil reaction and inhibition of clubroot.

Table 3.—*Effect of various soil treatments upon soil reaction, yield, and clubroot infection, Franksville plot, 1932*

Material applied	Rate of application per acre	Date of application	Elevation	Soil reaction, September 1932	Marketable heads	Clubroot infection	
						Severe	Slight
	Tons			*pH*	*Per cent*	*Per cent*	*Per cent*
None	0		High	6.9	0	100	0
	0		do	6.9	1	98	0
	0		Low	7.0	57	2	1
	0		High	6.9	1	98	0
	0		Low	7.2	68	2	4
Agstone	4	Oct. 22, 1930	High	6.3	1	98	0
	8	do	do	6.4	1	98	0
Calcium hydrate	1½	do	do	7.0	1	98	0
	1½	do	Low	7.3	49	16	8
	1½	Apr. 25, 1931	High	6.8	14	80	4
	1½	June 2, 1931	do	7.0	12	92	4
	2½	Oct. 22, 1930	do	7.0	1	98	0
	2½	do	Low	7.3	44	4	4
	2½	Apr. 25, 1931	do	7.2	57	4	8
	2½	June 2, 1931	do	7.2	52	0	0

The results of the field trials over a period of 3 years indicated that seasonal and soil factors influence greatly the action of liming materials as clubroot inhibitors. The remainder of this paper is concerned with the study of certain environmental factors in the greenhouse.

[9] Chupp, C. See footnote 3.

GREENHOUSE EXPERIMENTS

In pot experiments in the greenhouse, Wellman [10] found that as the chemicals were added to the soil in increasing amounts, infection was inhibited by $Ca(OH)_2$ when the pH reached 7.3, by $CaCO_3$ when it reached 7.9, but when K_2CO_3 was applied until the pH reached 8.1 infection still occurred. This experiment was repeated by the writers and certain other chemicals were included. Untreated soil from the Miller field was used. The experiment was run in three replications in 2-gallon earthenware crocks. The soil moisture was held at 75 to 80 percent of the water-holding capacity. The results are given in table 4.

TABLE 4.—*Effect of application of various chemicals to clubroot-infested soil upon soil reaction and disease development in pots in the greenhouse*

Chemical applied	Rate of application per acre	Series 1			Series 2			Series 3		
		Soil reaction	Healthy plants	Diseased plants	Soil reaction	Healthy plants	Diseased plants	Soil reaction	Healthy plants	Diseased plants
	Tons	*pH*	*Number*	*Number*	*pH*	*Number*	*Number*	*pH*	*Number*	*Number*
Commercial Agstone	1	5.7	0	20	5.8	0	20	5.7	0	20
	2	6.9	3	17	7.0	5	15	6.9	4	16
	4	7.2	20	0	7.2	20	0	7.3	20	0
	8	7.6	20	0	7.5	20	0	7.7	20	0
$CaCO_3$	1	5.5	0	20	5.6	0	20	5.9	0	20
	2	6.9	13	7	7.0	8	12	7.0	9	11
	3	7.0	20	0	7.3	20	0	7.2	20	0
$MgCO_3$	1	6.7	4	16	6.9	6	14	6.9	8	12
	2	7.1	18	2	7.3	17	3	7.2	20	0
	3	7.3	20	0	7.3	20	0	7.2	20	0
Commercial calcium hydrate	1	6.9	3	17	7.1	4	16	7.0	6	14
	2	7.2	20	0	7.4	20	0	7.3	20	0
	3	7.4	20	0	7.4	20	0	7.6	20	0
$Ca(OH)_2$	1	6.8	2	18	7.1	4	16	7.0	5	15
	2	7.2	20	0	7.3	20	0	7.3	20	0
	3	7.6	20	0	7.4	20	0	7.3	20	0
CaO	1	7.1	20	0	7.1	20	0	7.2	20	0
	2	7.3	20	0	7.3	20	0	7.3	20	0
	3	7.8	20	0	7.4	20	0	7.4	20	0
Na_2CO_3	1	5.6	0	20	5.8	0	20	5.8	0	20
	2	5.9	0	20	6.1	0	20	6.1	0	20
	3	6.1	0	20	6.4	0	20	6.3	0	20
K_2CO_3	1	5.7	0	20	5.7	0	20	5.9	0	20
	2	5.9	0	20	5.8	0	20	6.1	0	20
	3	6.1	0	20	6.0	0	20	6.3	0	20
Na_2SO_4	1	5.8	0	20	5.6	0	20	5.6	0	20
	2	6.1	0	20	6.0	0	20	5.8	0	20
	3	6.4	0	20	6.1	0	20	6.1	0	20
Untreated soil	0	5.7	0	40	5.6	0	40	5.6	0	40

Na_2CO_3, K_2CO_3, and Na_2SO_4, in the amounts used, raised the pH only slightly and did not affect clubroot infection. Commercial Agstone, finely ground dolomitic limestone, distinctly inhibited infection when the soil reaction reached pH 6.9 and completely prevented infection at pH 7.2 and 7.6. $CaCO_3$ gave similar results, while $MgCO_3$ was not completely effective until the soil reaction reached pH 7.3. These results differ from those of Wellman, who did not secure complete inhibition with $CaCO_3$ until the pH reached 7.9. $Ca(OH)_2$ produced little effect at pH 6.8 and 6.9 but was completely effective at 7.2 and above, while CaO prevented infection at 7.1.

[10] WELLMAN, F. L. See footnote 2.

It may be seen in this experiment that in the greenhouse those chemicals that were applied in amounts sufficient to raise the soil reaction slightly above neutral were in general very effective in preventing infection. This is in direct contrast to the results of field experiments described above in which the application of some of the same materials in quantities which raised the pH to 7.0 to 7.8 usually failed to prevent an abundant development of disease. As was pointed out earlier, in the Miller plot in 1930 and in the Franksville plot in 1932, calcium hydrate was effective in low portions of the fields where the soil moisture was relatively higher and conditions for plant growth were more favorable. In the greenhouse experiments a reasonably constant and favorable soil moisture was maintained and here also the liming materials were effective.

In the autumn of 1930 soil was secured from the untreated area and from each of the areas which had received 2- and 4-ton applications of calcium hydrate in the 1930 trials on the Miller plot. The reaction of the untreated soil was pH 5.8, that of the soil from the 2-ton area was pH 7.1, and that from the 4-ton area was pH 7.4. The three lots of soil were placed in 2-gallon earthenware jars and kept at 70 to 80 percent of the water-holding capacity. Twenty plants were grown in each sample for 1 month and the experiment was replicated at three different times. Heavy infection with clubroot was secured throughout the series in every plant on the untreated soil. No infection whatever occurred in the soil from the treated areas. In the autumn of 1931 soil was collected from the areas of the Miller plot which had received 4-ton and 8-ton applications of Agstone in October 1930. It may be recalled that in the 1931 season there had been no evidence of disease inhibition in these areas (table 1). At the time of collection the soil from the 4-ton Agstone area had a reaction of pH 7.4, and that from the 8-ton area had a reaction of pH 7.8. This soil was set up as described in the last experiment, and untreated soil was again used as a control. Forty plants were grown in each sample of soil for approximately 4 weeks. At the end of that time all plants in the untreated soil were severely diseased, none was diseased in the soil receiving the 8-ton treatment, while in that receiving the 4-ton treatment about 50 percent of the plants were infected. The experiment was repeated and the outcome was similar. These results gave conclusive evidence that the conditions surrounding the experiment in the greenhouse permitted complete control in the soil treated with 2-ton and 4-ton applications of calcium hydrate and an 8-ton application of Agstone, while conditions in the field had allowed heavy infection in the same soils. These results conform with those of other workers, who have secured consistent and convincing evidence of the effectiveness of lime in greenhouse tests while in the field various degrees of inconsistency have prevailed.

A study of this soil was continued by taking samples from the calcium-hydrate-treated areas over a period of about 2 years, and conducting greenhouse trials in the manner described above. The results are given in table 5. It is to be noted that as the interval after treatment increased the reaction of the treated soil gradually reverted to neutral and below. As the soil became more acid the percentage of infected plants gradually increased.

TABLE 5.—*Clubroot infection in field-treated soil when removed to the greenhouse at various intervals after treatment*

Treatment	Greenhouse tests with soil collected after—											
	2 months			12 months			17 months			23 months		
	pH	Plants used	Plants infected	pH	Plants used	Plants infected	pH	Plants used	Plants infected	pH	Plants used	Plants infected
		Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*		*Number*	*Percent*
Calcium hydrate, 2 tons per acre	7.1	70	0	7.0	70	18	6.8	70	68	6.4	70	90
Calcium hydrate, 4 tons per acre	7.4	70	0	7.3	70	0	7.0	70	0	6.7	70	40
Dolomitic hydrate, 2 tons per acre	7.2	70	0	7.1	70	12	6.9	70	56	6.4	70	70
Dolomitic hydrate, 4 tons per acre	7.6	70	0	7.4	70	0	7.1	70	0	6.9	70	30
Untreated	5.8	70	100	5.8	70	100	5.6	70	100	5.6	70	100

In the 1930 and 1931 field trials the soil moisture was relatively low for a major portion of the time, while in the greenhouse experiments it was kept consistently high. In order to ascertain whether soil moisture was a determining factor in the effectiveness of lime, soil from both 2-ton treatments with calcium and dolomitic hydrate in the 1930 Miller plot was set up at fairly constant soil-moisture levels of about 40 to 50 percent, 55 to 65 percent, and 70 to 80 percent of the water-holding capacity. Plants were transplanted to the crocks and the moisture levels maintained for 5 weeks. At the end of that time the plants in the untreated soil were uniformly infected at all moisture levels. In treated soils there was complete inhibition of infection at all moisture levels. The experiment was extended to the 8-ton Agstone-treated soil from the 1931 Miller plot. This soil was held at moisture levels of 40 to 50, 50 to 60, 60 to 70, 70 to 80, and 80 to 90 percent of the water-holding capacity. After 4 weeks all plants on the untreated soil held at 70 to 80 percent of the water-holding capacity were diseased. There were no diseased plants in the Agstone-treated soil at any of the moisture levels. The entire experiment was repeated with identical results.

Although the effectiveness of the two types of hydrate and Agstone as inhibitors of infection was uniform over a range of relatively constant soil-moisture levels, the conditions which prevailed still did not simulate those in the field, where there is usually a fluctuation of the moisture content of the soil. The next experiment was set up to observe the effect on infection of fluctuation in soil moisture.

Untreated soil and soil from the 2-ton treatment with calcium hydrate in the 1930 Miller plot were used in the winter of 1930–31. Two earthenware crocks were filled with the calcium hydrate field-treated soil and two with untreated soil. Ten plants were set in each crock and the moisture content of the soil was held at 70 to 80 percent of the water-holding capacity. Another lot of the treated soil was allowed to dry out until the moisture content was below 16 percent of the water-holding capacity. Four flats 20 inches long, 14 inches wide, and 12 inches deep were filled to a depth of 9 inches. Six

plants were set in each flat, each plant being watered in with 30 cc of water. After 5 days 50 cc of water was added to each flat by spraying it uniformly over the surface. After another 5 days 100 cc was added in the same manner and this procedure was repeated every 5 days during the remainder of the 6 weeks' duration of the experiment. Thus, in the earthenware crocks the soil moisture was quite constant and relatively high throughout the experiment. In the flats, however, the conditions resembled more closely those in the field trials, where plants were watered into relatively dry soil. The high moisture content of the soil around such transplants, besides being sufficient for infection, stimulated emergence of new secondary roots, which soon grew into the soil. The moisture content of this soil varied considerably from day to day and at different distances from the surface.

After 6 weeks the plants from all crocks and flats were removed and the experiment was repeated. The results of both series are given in table 6. In the crocks in which the moisture content was kept reasonably constant the results of previous experiments were duplicated. Every plant in the untreated soil was infected, but there was no infection in the treated soil. On the other hand, in the flats in which the moisture around the plants was relatively high at the time of transplanting, but was allowed to fluctuate in the whole bulk of the soil, every plant was infected. It is true that the average soil moisture in the flats was lower than in the same soil in the crocks, but infection cannot be attributed to low soil moisture alone since in previous experiments constant low soil moisture failed to favor infection. It appears that the fluctuation of soil moisture at relatively lower levels provided the proper conditions for infection in spite of the fact that calcium hydrate had been incorporated in the soil. Under these conditions the organism infected the plants readily as it had done earlier in the 1930 Miller plot.

TABLE 6.—*Clubroot infection in calcium-hydrate-treated and in untreated soil at constant and fluctuating soil moisture*

Treatment	Condition of soil moisture	Series 1		Series 2	
		Plants used	Plants infected	Plants used	Plants infected
		Number	*Percent*	*Number*	*Percent*
Untreated	Held at 70 to 80 percent of the water-holding capacity in crocks.	20	100	20	100
Calcium hydrate, 2 tons per acre.	do	20	0	20	0
Do	Started at 16 percent of the water-holding capacity, and allowed to fluctuate in flats.	24	100	24	100

Similar lots of treated and untreated soil were next set up in 2-gallon earthenware crocks at 70 to 80 percent of the water-holding capacity. The untreated soil and part of the crocks containing treated soil were held at constant high soil moisture by daily weighing and addition of water. The remaining crocks of treated soil were allowed to dry out gradually until the soil moisture approached 40 percent of the water-holding capacity. They were then brought up to the original moisture content by the addition of water. This

process was repeated twice during an interval of 4 weeks. When the plants were removed and examined all those in the untreated soil were diseased. In the treated soil no infection occurred where the moisture was held constant nor where the moisture level fell and rose periodically. Thus this type of fluctuation had the opposite effect from that in which fluctuation at low soil moisture occurred.

Another experiment was conducted to determine the effect of aeration of treated soil upon infection. Two-ton calcium-hydrate-treated soil was placed in 2-gallon earthenware crocks and a perforated soft lead tube was incorporated in the soil. By this means a continuous flow of air was forced through the soil. An equal number of nonaerated crocks were used. During the course of the experiment the soil moisture fluctuated from 65 to 80 percent of the water-holding capacity. After 30 days the plants were removed and the roots examined. The results of two series of experiments, given in table 7, show that the introduction of air into the treated soil resulted in 35 to 40 percent infection, while in nonaerated treated soil only 1 plant in 40 became infected. In untreated nonaerated soil all plants were severely diseased.

TABLE 7.—*Effect of aeration of calcium-hydrate-treated soil upon clubroot infection*

Soil treatment	Aeration	Series 1		Series 2	
		Plants used	Plants infected	Plants used	Plants infected
		Number	*Percent*	*Number*	*Percent*
Calcium hydrate, 2 tons per acre	Aerated	20	35	20	40
	Nonaerated	20	5	20	0
Untreated	do	20	100	20	100

DISCUSSION

Under the climatic conditions of 1930 to 1932, inclusive, hydrated lime did not uniformly inhibit clubroot infection in the field, although the soil reaction was in many cases changed to pH 7.0 or higher. However, when the same soil was removed to the greenhouse and plants were grown in it, the treatment was completely effective in preventing infection. Under the latter condition, where frequent watering was carried out, calcium hydrate, calcium carbonate, calcium oxide, and magnesium carbonate all reduced infection perceptibly when added in amounts sufficient to raise the pH to about 7.0, and usually inhibited infection completely at pH 7.2 or above. High or low relatively constant soil moisture did not change the degree of inhibition. Fluctuation of soil moisture and forced aeration, however, did permit varying degrees of infection in soil. It appears that fluctuation in soil moisture is likely to permit infection in slightly alkaline field soils.

While this study does not explain the fundamental bases of the effectiveness of lime as a clubroot inhibitor under various conditions, the results do show significant differences between greenhouse and field trials. It is obvious that greenhouse pot tests are not a true index of what may be expected in the field. If one were to consider

the greenhouse results given in table 4 alone there is ground for concluding that a distinct correlation exists between soil reaction and infection by the clubroot organism. Why this does not hold in the field is not explained, but it is clear that when the soil moisture fluctuates at relatively low levels abundant infection may occur at soil-reaction values which give no infection under the conditions of the experiments recorded in table 4.

It is suggested in this connection that the soil-reaction determination as ordinarily made is not necessarily a true index of the actual pH value of the soil closely adjacent to the roots. Thom and Humfeld [11] studied the reaction of soil particles adherent to fibrous root from a number of crop plants grown in a range of acid and alkaline soils. The soil in this zone, compared with the soil mass as a whole, was less acid when an acid soil was used, and less alkaline when an alkaline soil was used. It is possible that in the slightly alkaline treated soil employed in this study, the reaction in the immediate vicinity of the roots was sufficiently acid, under field conditions, to permit abundant infection. It is evident that treatment of soil sufficient to inhibit infection in the greenhouse in no sense eliminates the organism. When the reaction of such a soil naturally reverts to below pH 7.0 abundant infection occurs even when favorable soil moisture is maintained (table 5).

These results are offered only as they apply to the two soil types used. It is quite possible that heavier soils, higher in water-holding capacity, would yield entirely different results.

SUMMARY

Field treatments of Carrington silty clay loam and Clyde silty clay loam with calcium hydrate and calcium magnesium carbonate which raised the reaction to pH 7.1 and above did not generally inhibit clubroot development.

In treated soils, removed to the greenhouse, cabbage plants remained free from infection while in untreated soil from the same field there was abundant disease development.

Under greenhouse conditions infection was inhibited in treated soil at high, intermediate, and low relatively constant moisture levels.

Calcium hydrate, calcium carbonate, calcium oxide, and magnesium carbonate all reduced infection perceptibly in well-watered soil when added in sufficient amounts to raise the reaction of an acid soil to about pH 7.0, and usually inhibited infection completely at pH 7.2 or above.

Fluctuation of soil moisture at a relatively low soil-moisture level and forced aeration of the soil permitted varying degrees of infection in treated soils.

It is suggested that low fluctuating soil moisture is an influential factor in limiting the effectiveness of lime as a clubroot inhibitor in the field.

[11] THOM, C., and HUMFELD, H. NOTES ON THE ASSOCIATION OF MICROORGANISMS AND ROOTS. Soil Sci. 32: 29-36. 1932.

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